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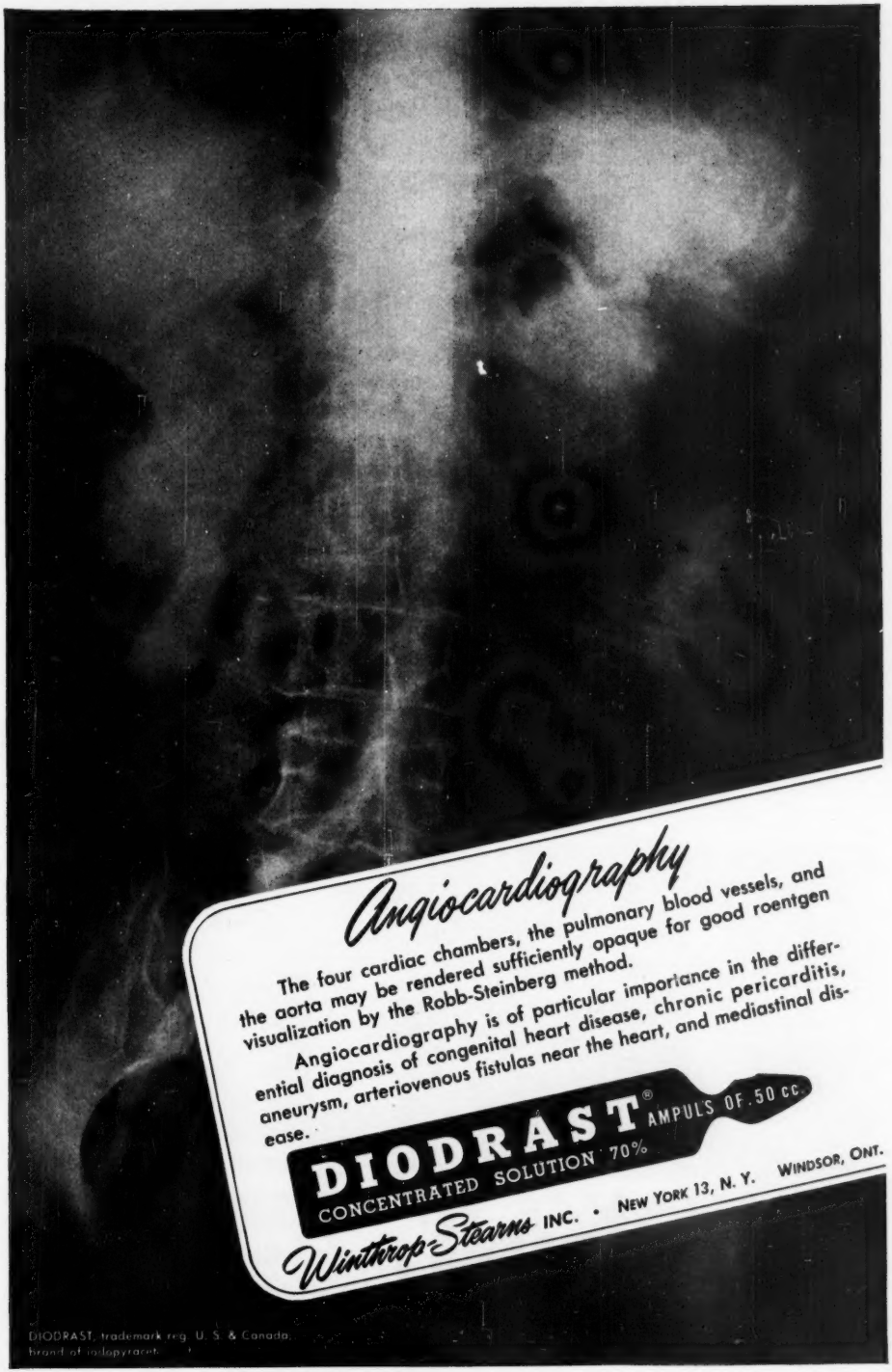

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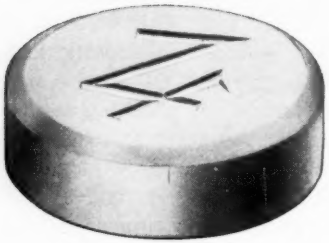

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THE EFFECT OF HEMOGLOBIN ON RENAL FUNCTION IN THE HUMAN

By JOHN H. MILLER AND ROGER K. McDONALD[1]

(From the National Heart Institute, National Institutes of Health, Bethesda, Md.; and the Gerontology Section, Baltimore City Hospitals, Baltimore, Md.)

(Submitted for publication May 16, 1951; accepted July 9, 1951)

It has long been noted that the occurrence of hemoglobinuria is frequently associated with oliguria, anuria, and renal failure. The cause of this impairment of renal function which accompanies the excretion of hemoglobin has been variously ascribed to obstruction of tubular lumina by precipitated hemoglobin or hemoglobin derivatives (1, 2), to renal ischemia associated with hemoglobinemia (3), or to a toxic action of hemoglobin on the kidney (4). More recently it has been postulated that the renal damage associated with the excretion of hemoglobin or heme containing substances is not due primarily to hemoglobin, but is caused by renal ischemia on the basis of shock (5, 6), which is frequently present in conditions under which hemoglobinuria occurs, *e.g.*, intravascular hemolysis following mismatched transfusion, the crush syndrome, and blackwater fever. This latter viewpoint holds that the presence of heme pigments in the plasma and urine is merely an incidental finding (6). It has been supported by a number of reports on the intravenous administration of hemoglobin solutions to the human (7–12) which generally agree that hemoglobin can be administered without untoward reaction, although no data on acute renal functional studies have been reported. Recently, however, Amberson and associates (13) have reported late and persistent decreases in renal clearances in a single hypertensive subject following the injection of hemoglobin.

Thus, not only has the mechanism of renal failure which frequently accompanies hemoglobinuria not been conclusively demonstrated, but the presence or absence of a deleterious effect of hemoglobin on the human kidney has not been clearly established.

During the course of a study of the glomerular permeability to hemoglobin, it soon became apparent that the administration of hemoglobin solutions to the human was attended by noteworthy alterations in renal function. The present report is concerned with observations on changes in renal function induced by the presence of extracorpuscular hemoglobin.

METHODS

Twenty-five male subjects who ranged in age from 24 to 65 years were selected for the present study from the wards of the Baltimore City Hospitals and the Baltimore City Infirmary. All subjects were adjudged to be free of cardiovascular and renal disease on the basis of the following criteria: (*1*) diastolic blood pressure below 90 mm. Hg, (*2*) absence of evidence of valvular heart disease or of cardiac enlargement, (*3*) absence of proteinuria in morning specimen by the heat-acetic acid test, (*4*) absence of abnormal findings on microscopic examination of sediment of freshly voided urine, (*5*) plasma nonprotein nitrogen below 35 mg. per cent, and (*6*) glomerular filtration rate and effective renal plasma flow within two standard deviations of the mean value for the subject's age group (14).

All subjects were studied in the fasting state. Approximately one liter of water was given by mouth during the two-hour period preceding the test; thereafter, 200 cc. of water were given at half-hour intervals during the procedure. The oral administration of sodium bicarbonate in a dosage of 2 gm. every four hours was instituted at approximately 18 hours before the test and was continued throughout the procedure.

The renal clearances of inulin and of p-aminohippurate (PAH) were determined before and after the intravascular administration of hemoglobin according to the methods of Goldring and Chasis (15).

In all experiments blood was sampled at the midpoint of each urine collection period through an indwelling needle in the femoral artery. Urine was obtained through a multi-eyed urethral catheter, and each urine collection period was terminated by washing the bladder with 100 cc. of physiological saline. Complete evacuation was assured by subsequent injection of air. Blood pressure, which was determined sphygmomanometrically, and pulse rate were measured twice during each urine collection period.

Inulin was determined in plasma and urine by the method of Harrison (16). PAH was determined in cadmium sulfate filtrates of plasma and in diluted urine by the method of Bratton and Marshall (17).

[1] Present address: U. S. Public Health Service Hospital, Wyman Park Drive, Baltimore 11, Maryland.

TABLE I

Renal functional changes following the intravascular administration of autogenous hemolyzed blood. Mean values for a group of 20 subjects*

	Control†	Period 4	Period 5	Period 6	Period 7
Inulin clearance (*cc./min.*)	107	85	81	81	79
PAH clearance (*cc./min.*)	576	285	299	307	321
Filtration fraction	.189	.310	.275	.270	.248
Urine flow (*cc./min.*)	5.8	2.4	3.0	3.3	3.1
Unresorbed filtrate (*per cent*)	5.2	2.9	3.6	3.9	3.8

* 12–15 gm. of hemoglobin administered over a period of 15 minutes. Periods 4–7 represent consecutive 20-minute urine collection periods after the appearance of hemoglobinuria.

† Mean of three 12-minute urine collection periods. Values not corrected for body surface area.

Plasma hemoglobin determinations were carried out according to the method of Evelyn and Malloy (18). The subjects were divided into three groups according to experimental design.

Group I. ($N = 20$). Following three 12-minute control urine collection periods, autogenous blood, freshly hemolyzed in distilled water was injected intravascularly. This hemoglobin solution was prepared by drawing 15 cc. of blood from the subject into a syringe containing 35 cc. of distilled water with a few drops of heparin. During the 36 minute control period, a total of 90 to 105 cc. of blood was withdrawn in this fashion, yielding 300 to 350 cc. of hemoglobin solution. This quantity of solution which contained an estimated 12–15 gm. of hemoglobin, assuming that complete or nearly complete hemolysis occurred, was then injected over a period of approximately 15 minutes. A few minutes after the appearance of hemoglobinuria, urine was collected in four consecutive periods, each of 20 minutes duration. In four of the subjects in this group, a radiopaque intravenous catheter was passed from an antecubital vein into the right renal vein under fluoroscopic control, and the fraction of PAH extracted from the plasma by the kidney was determined by simultaneous sampling of peripheral arterial and renal venous blood during control periods and following the injection of hemoglobin.

Group II. ($N = 10$). Following three control urine collection periods, 12 to 14 gm. of hemoglobin in the form of a 7.1 per cent commercially prepared solution [2] was injected intravascularly over a period of eight to ten minutes. Subsequent to the appearance of hemoglobinuria, urine was obtained in five consecutive 20-minute collection periods.

Group III. ($N = 6$). Following three 12-minute control urine collection periods, an intravenous infusion of either a 6.05 or 7.1 per cent hemoglobin solution (Sharp and Dohme) was instituted at the rate of approximately 1 cc. or 60 to 70 mg./min. Urine was obtained serially in collection periods of 15 minutes duration over a period of two to three hours, during which time the infusion of hemoglobin was maintained, and subsequently for an additional period of 60 to 90 minutes after discontinuance of the infusion.

Following 18 of the 36 experiments, determinations of the clearances of inulin and PAH were repeated at 24 or 48 hours after the injection of hemoglobin.

RESULTS

The injection of either the autogenous hemolyzed blood preparation or the commercially prepared hemoglobin solution was productive of the following physiological changes, all of which were statistically significant [3]: (*1*) an increase in the systolic and diastolic blood pressures, (*2*) a decrease in the pulse rate, (*3*) a decrease in the clearance of inulin and in the clearance of PAH, (*4*) an increase in the filtration fraction, (*5*) a decrease in the urine flow, and (*6*) an increase in the per cent of glomerular filtrate reabsorbed by the renal tubules.

Group I. Following the injection of autogenous hemolyzed blood, the mean systolic blood pressure increased from 126 mm. Hg during the control periods to 143 during the first postinjection period; the mean diastolic pressure increased from 78 to 93; the mean pulse pressure increased from 48 to 50; while the mean pulse rate decreased from 67 during control periods to 56 during the first postinjection period. Blood pressure remained significantly elevated and pulse rate remained significantly decreased during the entire 80-minute postinjection observation period.

The changes in renal clearances and in urine flow are shown in Table I. The mean depression of C_{PAH} during the first postinjection period was

[2] The hemoglobin solution employed in the present study was kindly supplied by Sharp and Dohme, Inc., Glenolden, Pa. The preparation is a stable solution of reduced human hemoglobin, free of red cell stroma and of electrolyte content approximating that of plasma (19).

[3] The term "significant" in the present report is used to indicate statistical significance at the 1 per cent level of confidence as determined by the t-test of Fisher.

50.1 per cent, while the mean depression of C_{IN} was 20.8 per cent. The clearances of both PAH and inulin remained significantly depressed during the entire 80-minute postinjection period. The filtration fraction was increased from a control value of 0.189 to 0.310 during the first postinjection period and remained significantly elevated throughout the 80-minute postinjection period.

The control urine flow of 5.8 cc./min. decreased to 2.4 cc./min. during the first postinjection period and remained significantly depressed throughout the 80-minute observation period. The per cent of filtrate unresorbed by the tubules likewise decreased from a control value of 5.2 per cent to 2.9 per cent during the first postinjection period. The percentage reabsorption of filtrate during the last period (*i.e.*, 60–80 minutes after the injection of hemoglobin) did not differ significantly, however, from the control value.

It can be seen in Table II that the renal extraction of PAH following the injection of hemoglobin was essentially unchanged in three of four subjects. This observation indicates that the clearance of PAH remained a valid measure of renal plasma flow, and that the observed depression in PAH clearance was due to an actual decrease in renal plasma flow rather than to an impairment of tubular function. In subject J. B., the renal extraction of PAH of 0.934 during the control periods decreased to 0.732 and to 0.681 following the injection of hemoglobin. This depression may have been due either to impairment of tubular function or to displacement of the tip of the catheter to the orifice of the renal vein. Regardless of the cause of the depression, it should be noted that the per cent decrements in C_{PAH} and in true renal plasma flow remain in fairly close agreement.

Group II. Following the rapid injection of the commercially prepared hemoglobin solution at a rate of approximately 1.5 gm./min., systolic blood pressure increased from a mean control value of 107 mm. Hg to 140 during the first postinjection period; diastolic pressure increased from 76 to 98; pulse pressure increased from 31 to 42; and the mean pulse rate decreased from 64 during control periods to 56 during the first postinjection period. As was the case in the group receiving an injection of hemolyzed blood, there was subsequently no significant change in blood pressure or pulse rate during the 100-minute postinjection period.

C_{PAH} was depressed 62 per cent for the group during the first postinjection period and, although plasma flow was still significantly depressed from control levels during the last period (*i.e.*, 80 to 100 minutes), it had shown a significant increase from the fourth period in a return toward baseline levels. Glomerular filtration rate was decreased 37 per cent during the first postinjection period and, as was the case with C_{PAH}, although it was still significantly depressed at 100 minutes, it had shown a significant increase toward baseline levels. The filtration fraction, which was elevated throughout the experimental period, showed a significant

TABLE II

*Renal extraction of PAH following hemoglobin**

Subject	Function	Control	Period 4	*per cent change*	Period 5	*per cent change*	Period 6	*per cent change*
E. H.	PAH Ext.	.905	.911		.909		.918	
	C_{PAH} (*cc./min.*)	475	231	−51.4	292	−38.6	314	−33.9
	RPF (*cc./min.*)	525	254	−51.7	321	−38.9	342	−34.9
J. F.	PAH Ext.	.968	.953					
	C_{PAH} (*cc./min.*)	772	229	−70.4				
	RPF (*cc./min.*)	798	240	−70.0				
J. A.	PAH Ext.	.939	.918		.874			
	C_{PAH} (*cc./min.*)	458	64	−86.0	106	−76.9		
	RPF (*cc./min.*)	488	70	−85.7	121	−75.3		
J. B.	PAH Ext.	.934	.732		.681			
	C_{PAH} (*cc./min.*)	590	126	−78.7	226	−61.7		
	RPF (*cc./min.*)	632	172	−72.8	332	−47.5		

* 12–15 gm. of hemoglobin administered over a period of 15 minutes.

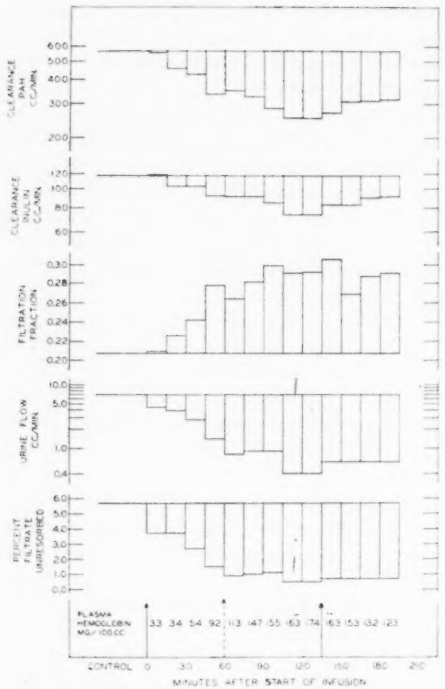

FIG. 1. RENAL FUNCTIONAL CHANGES DURING THE INTRAVENOUS INFUSION OF A COMMERCIALLY PREPARED HEMOGLOBIN SOLUTION AT A RATE OF 70 MG./MIN.

Control values represent the mean of three urine collection periods each of 12 minutes duration. Solid arrows represent the beginning and end of the hemoglobin infusion. Broken arrow represents the point at which hemoglobinuria occurred.

decline from period 4 to period 8 toward control levels (Table III).

The mean control urine flow of 6.1 cc./min. decreased to 1.9 cc./min. during the first period following the injection of hemoglobin. Although urine flow was still significantly decreased (3.9 cc./min.) during period 8, it increased progressively toward baseline levels throughout the 100 minute observation period. The mean per cent of filtrate unresorbed by the tubules decreased from a control value of 5.2 per cent to 2.5 per cent during the first postinjection period, and as was the case with hemolyzed blood, tubular reabsorption of filtrate during period 8 was no longer significantly elevated from control levels (Table III).

Group III. The administration of hemoglobin solution at a rate of approximately 60 to 70 mg./min. was productive of a progressive decrease in renal plasma flow and glomerular filtration rate, a progressive increase in blood pressure and filtration fraction, and a progressive decrease in urine flow. A typical infusion experiment is represented in Figure 1. It should be noted that changes in urine flow and in renal hemodynamics occurred during the first urine collection period, when as little as a few hundred milligrams of hemoglobin had been infused. Following cessation of the infusion, there was a gradual return of plasma flow, filtration rate, urine flow, and blood pressure toward baseline values.

In follow-up clearance studies performed in 18 of the 36 experiments at 24 to 48 hours after the injection of hemoglobin, the clearances of inulin and PAH did not differ significantly from the pre-hemoglobin control clearance values.

DISCUSSION

It has been suggested that the untoward effects which may accompany the injection of hemoglobin preparations in the human or in the experimental animal may be due not to hemoglobin *per se*, but to the presence of other factors such as red cell stroma, potassium released from the red cell, organic solvents employed as laking agents, bacterial contamination of the solution, or toxic derivatives of hemoglobin, such as, methemoglobin (11, 12, 20). Although the agent effective in producing the alterations in renal function observed in the present study has not been clearly identified, it seems most likely that either hemoglobin *per se* or some product of its intravascular catabolism was responsible.

Qualitatively, the same renal functional changes occurred with both the hemolyzed blood preparation and the commercially processed solution. Neither bacterial contamination nor organic laking agents were present in either of these solutions; nor could the presence of methemoglobin be demonstrated spectrophotometrically in either preparation. Although red cell stroma, and potassium in excess of normal plasma concentration were present in the hemolyzed blood preparation, these factors were both absent in the commercially prepared hemoglobin solution.

TABLE III

*Renal functional changes following the intravascular administration of a commercially prepared hemoglobin solution**

Subject	Function	Control†	Period 4	Period 5	Period 6	Period 7	Period 8
R. K.	Inulin clearance (*cc./min.*)	80	32	44	47	50	44
	PAH clearance (*cc./min.*)	399	81	123	159	201	194
	Filtration fraction	.205	.395	.358	.296	.249	.227
	Urine flow (*cc./min.*)	1.4	0.5	0.8	1.0	1.5	1.5
	Unresorbed filtrate (*per cent*)	1.7	1.6	1.8	2.1	3.0	3.4
R. H.	Inulin clearance	117	60	54	55	75	72
	PAH clearance	673	221	222	255	329	338
	Filtration fraction	.174	.271	.243	.216	.228	.213
	Urine flow	11.5	1.5	1.7	2.0	3.6	4.6
	Unresorbed filtrate	9.8	2.5	3.1	3.6	4.8	6.4
W. P.	Inulin clearance	133	83	96	84	99	87
	PAH clearance	727	280	367	323	386	351
	Filtration fraction	.183	.296	.262	.260	.256	.248
	Urine flow	3.1	1.4	2.3	1.6	1.8	1.5
	Unresorbed filtrate	2.3	1.7	2.4	1.9	1.8	1.7
J. W.	Inulin clearance	118	72	72	72	73	74
	PAH clearance	669	304	299	299	304	344
	Filtration fraction	.176	.237	.241	.241	.240	.215
	Urine flow	1.6	1.1	2.9	2.9	1.8	2.2
	Unresorbed filtrate	1.4	1.5	4.0	4.0	2.5	3.0
W. L.	Inulin clearance	160	72	78	74	89	98
	PAH clearance	1008	293	366	429	511	534
	Filtration fraction	.159	.246	.213	.172	.174	.184
	Urine flow	4.8	1.2	2.1	2.5	3.4	4.2
	Unresorbed filtrate	3.0	1.7	2.7	3.4	3.8	4.3
F. B.	Inulin clearance	99	60	75	80	78	76
	PAH clearance	664	201	298	352	395	386
	Filtration fraction	.150	.298	.252	.227	.197	.197
	Urine flow	9.0	1.2	2.4	3.4	4.0	4.6
	Unresorbed filtrate	9.1	2.0	3.2	4.2	5.1	6.0
E. H.	Inulin clearance	87	72	69	66	64	69
	PAH clearance	560	278	253	252	250	278
	Filtration fraction	.156	.259	.273	.262	.256	.248
	Urine flow	4.5	2.8	2.8	2.6	2.8	3.0
	Unresorbed filtrate	5.2	3.9	4.0	3.9	4.4	4.3
H. H.	Inulin clearance	140	104	100	96	109	113
	PAH clearance	711	324	373	377	436	433
	Filtration fraction	.196	.321	.268	.255	.250	.261
	Urine flow	9.9	3.5	4.2	5.0	7.0	7.2
	Unresorbed filtrate	7.1	3.4	4.2	5.2	6.4	6.4
P. N.	Inulin clearance	117	72	68	73	82	91
	PAH clearance	655	289	271	304	345	361
	Filtration fraction	.179	.249	.251	.240	.238	.252
	Urine flow	5.4	2.8	2.4	2.8	4.3	4.7
	Unresorbed filtrate	4.8	3.9	3.5	3.8	5.2	5.2
W. T.	Inulin clearance	137	125	114	140	124	129
	PAH clearance	829	366	367	454	451	423
	Filtration fraction	.166	.342	.311	.308	.275	.305
	Urine flow	10.1	3.1	3.8	2.6	2.9	5.9
	Unresorbed filtrate	7.6	2.5	3.3	1.8	2.3	4.6
Mean	Inulin clearance	119	75	77	79	84	85
	PAH clearance	690	264	294	320	361	364
	Filtration fraction	.174	.291	.267	.248	.236	.235
	Urine flow	6.1	1.9	2.5	2.6	3.3	3.9
	Unresorbed filtrate	5.2	2.5	3.2	3.4	3.9	4.5

* 12–14 gm. of hemoglobin administered over a period of 8–10 minutes. Periods 4–8 represent consecutive 20-minute urine collection periods after the appearance of hemoglobinuria.

† Mean of three 12-minute urine collection periods. Values not corrected for body surface area.

Comparing the responses to the single injection of hemolyzed blood (Group I) and to the single injection of commercial hemoglobin solution (Group II), it will be noted that the fall in PAH clearance, expressed as either the absolute or per cent decrement, was significantly greater after commercial hemoglobin. Blood pressure increased in all subjects, somewhat more in Group II than in Group I. A reduction in renal vascular caliber, which was greater for the stroma-free preparation than for the hemolyzed blood, therefore, must have occurred. Although the total amount of hemoglobin administered was approximately the same for both solutions, the hemoglobin of the more concentrated commercial preparation was injected at approximately twice the rate of the hemoglobin of the hemolyzed blood preparation. The quantitative difference in the effect of the two preparations may well be explained on a time-dosage basis. In accord with this explanation is the fact that when the commercial preparation was infused slowly (Group III), the vasoconstrictive response was less marked.

The demonstration of a renal vasoconstrictive phenomenon in the human following the intravascular injection of hemoglobin is in accord with the findings of other observers who have measured renal blood flow in the dog by plethysmographic methods. Reid demonstrated that the intravenous injection of either distilled water or of autogenous blood laked with distilled water or ether was productive of an immediate decrease in renal volume (21, 22). Mason and Mann (23) likewise demonstrated a decrease in the renal volume in the dog following the injection of a hemoglobin solution, but noted no change in renal volume after the administration of a saline suspension of red cell stroma. Similar findings of renal vasoconstriction induced by hemoglobin were reported by Hesse and Filatov (3). A pressor effect in the human was noted by Amberson and associates (13), who reported an increase in blood pressure and a slowing of pulse rate following the injection of hemoglobin.

For many years the oliguria and renal failure which occur with hemoglobinuria were generally attributed to obstruction of the renal tubules by casts and debris of hemoglobin or its derivatives (1, 2, 24, 25), since such material was commonly found to be present in the tubules at autopsy. More recently, the significance of intraluminal deposition of hemoglobin substances in the production of anuria and renal failure has been questioned (5, 6). It has been pointed out that there is little correlation between the degree of tubular obstruction observed at autopsy and the degree of suppression of urine flow observed clinically, and that not infrequently, the degree of blockage observed at postmortem is insufficient to account for anuria (5).

Although the significance of the role of tubular obstruction by hemoglobin and its derivatives in the production of renal failure cannot be evaluated from the present study, the data suggest that renal damage associated with hemoglobinuria may be due primarily to renal ischemia on the basis of renal vasoconstriction induced by the presence of extracorpuscular hemoglobin. The intravascular administration of hemoglobin in dosages as low as 0.5 gm. in the human has been shown to result promptly in renal vasoconstriction. Although the dosages employed in the present study produced only a transient renal hemodynamic response, insofar as renal clearances had returned to baseline levels by 24 or 48 hours after the injection of hemoglobin, it is not inconceivable that a more intense or more prolonged hemoglobinemia might produce renal ischemia of sufficient intensity and duration to result in either reversible or irreversible renal damage.

Moreover, it must be remembered that shock is not infrequently present in conditions which result in the occurrence of hemoglobinuria or myoglobinuria. Renal vasoconstriction and decreased renal plasma flow have been described in both experimental (26) and clinical (27) shock. In such circumstances, the summation of the effects of shock and hemoglobinemia on the kidney may result in renal ischemia of intensity and duration sufficient to effect renal damage.

The clinical association of oliguria with the phenomenon of hemoglobinuria finds an interesting parallel in the present study. The presence of hemoglobin, free in the plasma, produced a prompt decrease in urine flow. The infusion studies demonstrated the occurrence of this phenomenon in the absence of hemoglobinuria at plasma levels well below the renal threshold for hemoglobin.

Two factors contribute to the oliguria following the injection of hemoglobin: a decrease in the rate of glomerular filtration and an increase in the per cent of filtrate reabsorbed by the renal tubules. Although the cause of this increased tubular reabsorption is not understood, it would not appear to be on the basis of unselective reabsorption of filtrate by damaged tubules as postulated by Van Slyke (*28*) as being responsible for the oliguria and anuria following shock, inasmuch as the maintainence of tubular integrity has been demonstrated in the present studies by an unchanged renal extraction of PAH following the administration of hemoglobin.

During the past decade considerable interest has been aroused in the possibility of utilizing hemoglobin solutions as a plasma or blood substitute (*9, 12, 29,* 30). It has been pointed out that such solutions would not only exert colloid osmotic pressure in the restoration of blood volume but would also possess the ability to transport oxygen. The demonstration in the present study of moderate to intense renal vasoconstriction produced by hemoglobin in amounts far less than would be used in the treatment of shock, seriously questions the feasibility of employing hemoglobin solutions for therapeutic purposes.

SUMMARY AND CONCLUSIONS

A total of 36 renal clearance studies was performed on 25 male subjects before and after the intravascular administration of hemoglobin in dosages ranging up to 15 gm.

The injection of either an autogenous hemolyzed blood preparation or a commercially prepared hemoglobin solution promptly resulted in the following physiological changes: (*1*) an increase in the systolic and diastolic blood pressures, (*2*) a decrease in the pulse rate, (*3*) a decrease in the clearances of inulin and PAH, (*4*) an increase in the filtration fraction, (*5*) a decrease in the urine flow, and (*6*) an increase in the per cent of glomerular filtrate reabsorbed by the renal tubules. The renal extraction of PAH at low plasma levels was essentially unchanged following the administration of hemoglobin.

In follow-up studies performed in 18 of the 36 experiments at 24 to 48 hours after the injection of hemoglobin, inulin and PAH clearances had returned to control levels.

It is suggested that renal vasoconstriction induced by the presence of extracorpuscular hemoglobin may play an important role in the production of renal damage associated with hemoglobinuric states.

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HUMAN GLOMERULAR PERMEABILITY AND TUBULAR RECOVERY VALUES FOR HEMOGLOBIN

By ROGER K. McDONALD,[1] JOHN H. MILLER, AND ELEANORE B. ROACH

(From the Section on Gerontology, National Heart Institute, National Institutes of Health, P.H.S., F.S.A., Bethesda, Md., and the Baltimore City Hospitals, Baltimore, Md.)

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Evidence for the glomerular filtration and tubular recovery of hemoglobin has been provided by several investigators (1–5). The simultaneous determination of hemoglobin and creatinine clearances in dogs and rabbits (2, 3) and of hemoglobin and inulin clearances in man has given ratios between 0.02 and 0.05. These values have been interpreted as the permeability of the glomeruli to hemoglobin relative to creatinine or inulin. Harrison's studies on dogs indicated the glomerular permeability to hemoglobin was between 4 and 20 per cent of the permeability to creatinine (5). The capacity of the renal tubules for hemoglobin recovery varied between 4 and 45 mg./100 cc. of glomerular filtrate.

The present study was undertaken primarily to determine the glomerular permeability and tubular recovery values for hemoglobin in the human kidney. In addition, the fact that hemoglobin and serum albumin may have approximately the same molecular weight, volume, and dissymmetry (6) suggested that the study of the glomerular permeability to hemoglobin might provide a means for determining the renal factors involved in the production of proteinuria.

METHODS

Subject selection criteria, the general test procedure, laboratory analyses and the preparation and administration of hemoglobin have been described previously (7). The subjects studied were placed into one of three groups depending upon the rate of administration of hemoglobin.

Rapid (N = 9). All subjects received the commercially prepared hemoglobin solution [2] in a single injection at a rate of about 1,400 mg./min. Six of the nine subjects were retested at intervals of two or more weeks to determine the reproducibility of the values obtained (Group II of reference 7).

Intermediate (N = 14). All subjects received an autogenous hemolyzed blood preparation in a single injection at a rate of about 700 mg. per minute (Group I of reference 7).

Slow (N = 6). The commercially prepared hemoglobin solution was administered by slow infusion at a rate of about 60–70 mg./min. Five of the six subjects were retested at intervals of two or more weeks to determine the reproducibility of values (Group III of reference 7).

In all three groups arterial plasma and urine hemoglobin concentrations were determined for each urine collection period (8).

RESULTS

The relationship between the rate of urinary excretion of hemoglobin (Y) to the plasma concen-

TABLE I

*Values for glomerular permeability (K), Tm_{Hb}, and T_{Hb} obtained with a single injection of commercially prepared hemoglobin**

Subject	Date	K	Tm_{Hb}	T_{Hb}
			mg. Hb/min.	*mg. Hb/100 cc. filtrate*
E. H.	2/28/50	.12	13.2	19.1
	6/6/50	.11	15.6	20.9
H. H.	2/1/50	.17	26.5	25.2
	3/30/50	.14	16.4	16.0
P. N.	1/4/50	.16	16.6	21.2
	1/25/50	.17	14.8	18.0
W. P.	12/7/49	.20	21.3	25.0
	1/21/50	.20	29.2	32.2
J. W.	12/7/49	.19	25.1	34.6
	3/7/50	.14	16.7	20.0
R. H.	11/21/49	.23	24.4	39.8
	1/19/50	.14	13.4	15.7
W. T.	3/1/50	.11	17.7	14.4
W. L.	2/14/50	.15	17.0	21.8
F. B.	12/14/49	.20	17.8	24.2
Mean		.162	19.04	23.21

* Each subject received 12 to 14 gm. of hemoglobin at an approximate rate of 1,400 mg./min.

[1] Present address: U. S. Public Health Service Hospital, Wyman Park Drive, Baltimore 11, Maryland.

[2] The hemoglobin solution used in part of this study was kindly provided by Sharp and Dohme, Inc., Glenolden, Pa.

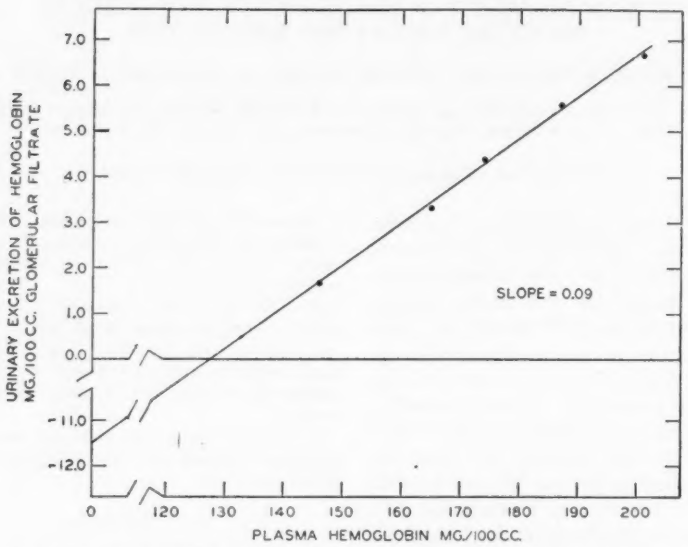

FIG. 1. THE RELATIONSHIP BETWEEN THE PLASMA HEMOGLOBIN CONCENTRATION AND THE URINARY EXCRETION OF HEMOGLOBIN/100 CC. OF GLOMERULAR FILTRATE

The X intercept of the linear function indicates the theoretical renal threshold. The Y intercept of the linear function indicates the tubular recovery of hemoglobin in mg./100 cc. of filtrate. Subject received 8.5 gm. of hemoglobin at a rate of 60 mg./min.

tration of hemoglobin (X) was rectilinear for all subjects (Y is expressed as urinary hemoglobin excretion in mg./100 cc. of glomerular filtrate; X as mg. hemoglobin/100 cc. plasma). Figure 1 shows the plot obtained for a single subject, E. H. This relation is of the form $Y = KX + T$, where the slope K indicates permeability of the glomeruli to hemoglobin relative to inulin. T_{Hb} is the Y intercept and represents the tubular component of hemoglobin excretion in mg./100 cc. of glomerular filtrate. T_{Hb} is negative, indicating that the tubules remove hemoglobin from the filtrate. T_{Hb}/K is the X intercept which represents the theoretical renal threshold for hemoglobin. Tm_{Hb} for the individual (expressed in mg. hemoglobin per minute), is obtained by multiplying the T_{Hb} in mg. hemoglobin/cc. glomerular filtrate by the average glomerular filtration rate (C_{IN}).

TABLE II

*Values for glomerular permeability (K), Tm_{Hb}, and T_{Hb} obtained with autogenous hemolyzed blood**

Subject	Date	K	Tm_{Hb}	T_{Hb}
			mg. Hb/min.	*mg. Hb/100 cc. filtrate*
R. B.	6/9/49	.07	3.1	5.7
S. D.	6/20/49	.08	8.4	13.0
E. K.	6/21/49	.16	18.0	29.2
T. B.	6/24/49	.13	14.1	16.5
A. V.	7/13/49	.12	8.2	11.2
A. B.	7/21/49	.10	12.2	12.9
J. S.	8/16/49	.14	14.0	19.2
P. D.	8/17/49	.20	20.8	28.8
C. S.	9/27/49	.10	20.8	16.5
E. H.	10/20/49	.10	13.2	18.1
P. P.	11/2/49	.10	4.3	7.1
W. P.	11/20/49	.10	11.8	10.2
P. N.	12/19/49	.20	17.6	22.2
W. L.	1/3/50	.15	20.1	16.9
Mean		.125	13.10	16.50

* Each subject received from 12 to 15 gm. of hemoglobin at an approximate rate of 700 mg./min.

The derived renal threshold values for groups designated as Rapid, Intermediate and Slow were 148.2 ± 42.0, 133.7 ± 31.0, and 96.4 ± 25.5 mg. of hemoglobin/100 cc. of plasma. The renal thresholds for the Rapid and Intermediate groups were significantly higher than that of the Slow group ($P < .01$).

The results obtained for K, T_{Hb}/100 cc. of filtrate and Tm_{Hb} are shown in Tables I, II, and III. The mean K (0.162) for the Rapid group (Table I) was significantly higher than the mean K of 0.101 for the Slow group ($P < .01$—Table III).

The mean Tm_{Hb} of 19.04 mg./min. for the group that received the single rapid injection of the commercial hemoglobin solution was significantly higher than the Tm_{Hb} for either the Intermediate group (13.10 mg./min.—Table II) or for the Slow group (9.07 mg./min.—Table III). During the periods of time in which our subjects were studied, the tubular recovery of hemoglobin was not only constant per unit of filtrate in a given individual throughout the experimental procedure but was also apparently operative at a maximum rate since it remained constant as the plasma level of hemoglobin changed and the load of hemoglobin presented for tubular recovery varied.

Although the graphic estimation of K and T_{Hb} gave highly reliable results when applied to the data of a single experiment,[3] it can be seen from Table II and III that the values obtained on different days in the same subject tended to vary widely. Indeed, duplicate tests on subjects P. N., W. P., and J. W. (Table III) differed to a greater extent than did any of their values from the group mean. The potential effect of differences in urine flow on the results of duplicate tests was determined and found to be considerably less than one per cent.

In several subjects, serial examination of the urine specimens obtained following the injection of hemoglobin revealed a microscopic hematuria of variable intensity but never sufficiently great to cause a significant alteration in the urine hemoglobin values.

[3] The correlation coefficient for values of K derived independently by two observers using the same experimental data was 0.98.

TABLE III

*Values for glomerular permeability (K), Tm_{Hb}, and T_{Hb} obtained with a slow infusion of commercially prepared hemoglobin solution**

Subject	Date	K	Tm_{Hb}	T_{Hb}
			mg. Hb/min.	*mg. Hb/100 cc. filtrate*
E. H.	1/30/50	.07	6.7	8.4
	5/10/50	.09	9.2	11.4
H. H.	2/23/50	.15	13.9	14.7
	4/13/50	.14	15.8	14.9
P. N.	2/15/50	.12	6.3	7.4
	3/6/50	.08	5.8	5.7
W. P.	1/31/50	.12	9.7	11.6
	2/20/50	.05	1.7	1.7
J. W.	1/25/50	.08	6.9	8.1
	2/14/50	.14	13.8	16.5
W. T.	2/8/50	.07	10.0	7.8
Mean		.101	9.07	9.84

* Each subject received hemoglobin at a rate of 60 to 70 mg./min.

DISCUSSION

The term glomerular permeability (K) is not used in the absolute sense but is used as the permeability of the glomerular bed to hemoglobin relative to its permeability to inulin. Other workers (2–4) have expressed relative permeability as the ratio of the clearance of hemoglobin to the clearance of creatinine in dogs or to the clearance of inulin in humans. This method fails to take into account the factor of tubular recovery. The ratio C_{Hb}/C_{IN} approaches the true permeability, $\frac{U_{Hb} + Tm_{Hb}}{P_{Hb}\, C_{IN}}$, asymptotically as the plasma hemoglobin concentration (P_{Hb}) increases. The plasma hemoglobin concentrations used in the present study were of the order of twice the renal threshold (200–300 mg./100 cc.) and the use of the clearance ratio method would have given K values for hemoglobin about half of the actual values obtained. Plasma hemoglobin values ten times the renal threshold (1000–1500 mg. per cent) would be required to give ratios equal to 90 per cent of the true permeability values. The results of our observations on humans are in much closer agreement with the canine values obtained by Harrison (5), who also derived the K values for hemoglobin in the manner shown in Figure 1, than they are with the values for human subjects obtained by Brandt, Frank, and Lichtman (4) using the clearance ratio method.

The glomerular permeability constants for hemoglobin obtained in the present study would be inordinately high if applied to plasma albumin, since approximately 0.5 to 1.0 gm. of protein would then be filtered each minute by the glomer-

uli of the normal human kidney.[4] Analysis of glomerular filtrate using a method sensitive to about 30 mg. per cent in the mammalian kidney has failed to demonstrate the consistent presence of protein (11, 12). In rabbits protein concentrations of 15 to 22 mg. per cent in the glomerular filtrate have been demonstrated (13). This protein concentration would suggest a permeability constant not greater than 0.005 for plasma albumin. Thus it is apparent that the glomerular permeability to hemoglobin obtained under the conditions of this study differs significantly from the permeability to plasma albumin in spite of the fact that both substances are structurally similar (6).

The pronounced decrease in glomerular filtration rate and renal blood flow resulting from the intravascular administration of hemoglobin (7) suggests an explanation for the high K values for hemoglobin obtained in this study. The decreases in renal blood flow were of sufficient magnitude to produce a marked ischemia in a number of glomeruli. That a change in permeability sufficient to allow a significant leakage of plasma protein out of capillaries can be produced by a few minutes of ischemia or anoxic anoxia has been demonstrated (14, 15). The observation that a delay period of four to 25 minutes elapsed between the end of the rapid injection of hemoglobin and the appearance of hemoglobinuria is consistent with an ischemia of some glomeruli with subsequent increased permeability when blood flow is reestablished. The fact that the glomerular permeability to hemoglobin was significantly higher when the individuals received a single rapid injection than when they received a slow infusion supports this speculation; for only with the rapid administration of hemoglobin was there an intense, initial renal vasoconstriction (7). The association of proteinuria with decreased glomerular filtration rate and renal blood flow has been observed by others (16, 17). Especially pertinent to this hypothesis is the observation of White and Rolf (16) that during heavy exercise a group of normal subjects developed a marked decrease in glomerular filtration rate and renal blood flow. Before and during the exercise the urine was free of protein, but urine formed in the first and second post-exercise periods (40 minutes after exercise) contained protein.

The variations in tubular recovery of hemoglobin noted between individuals and between repeat determinations on the same individual were associated with parallel changes in the glomerular permeability. This observation is also in accord with the concept that the filtration of hemoglobin was inordinately high in those glomeruli whose permeability characteristics were altered to some degree by ischemia. The greater the number of glomeruli filtering hemoglobin, the greater the number of tubules that would participate in recovery, and the greater would be the rate of tubular recovery as determined for the kidney as a whole.

The coexistence of high renal thresholds for hemoglobin and high glomerular permeability to hemoglobin is an apparent paradox. The term, renal threshold, is a value dependent upon three variables, *viz.*, glomerular permeability, glomerular filtration rate and tubular recovery. The presence of the highest renal thresholds in the subjects with the highest glomerular permeability to hemoglobin indicates that the tubular recovery is sufficiently high to prevent hemoglobinuria from occurring at the lower plasma levels. This finding may be interpreted to mean that with greater renal ischemia less filtrate is available per nephron filtering hemoglobin and the tubules are thus able to recover the filtered hemoglobin more completely.

Finally, it is suggested that on the basis of the findings of the present study, the intravascular administration of hemoglobin increases the glomerular permeability to protein. For this reason hemoglobin does not appear to be a satisfactory tool for the study of glomerular permeability in the normal or the diseased kidney.

SUMMARY AND CONCLUSIONS

The mechanism of hemoglobin excretion by the human kidney has been studied in 19 subjects. It is concluded that hemoglobin is filtered by the glomeruli and recovered by the tubules to a limited extent. The glomerular permeability to hemoglobin was significantly lower ($K = 0.101$) when

[4] It is possible that some degree of dissociation of the hemoglobin molecule might occur at the low plasma concentrations employed in this study (9, 10), but the absence of any quantitative information on this possibility precludes consideration here.

hemoglobin was administered at a slow rate (70 mg./min.) than when it was administered at a rapid rate (1,400 mg./min.) ($K = 0.162$). The permeability of the glomeruli to hemoglobin averaged 12.9 per cent of the glomerular permeability to inulin. The renal tubular recovery of hemoglobin averaged 17.1 mg. per 100 cc. of glomerular filtrate.

It is suggested that the intravascular administration of hemoglobin alters glomerular permeability. How this effect is mediated is unknown, but the sequence of renal vasoconstriction followed by ischemic alteration of glomerular capillaries seems likely.

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THE EFFECT OF KHELLIN ON CARDIO-PULMONARY FUNCTION IN CHRONIC PULMONARY DISEASE [1, 2]

By E. N. SILBER,[3] G. L. SNIDER, H. GOLDBERG, L. N. KATZ, AND D. B. RADNER

(From the Cardiovascular Department, Medical Research Institute, and the Chest Department, Michael Reese Hospital, Chicago, Ill.) [4]

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Extract from the fruit of the plant, Ammi Visnaga Lam, has long been employed by inhabitants of the Middle East for relief of renal colic. The major biologically active component, khellin, was isolated in impure form by Fantl and Salem in 1930 (1). The chemical structure of khellin (visammin) was established by Spath and Gruber (2), and the pharmacology of this compound was investigated by several groups in Egypt, chief among them being Samaan (3) and Anrep, Barsoum, and Kenawy (4). These investigators have reported that khellin exhibits marked coronary dilator, bronchodilator and antispasmodic activity.

Reports of beneficial results from its use in angina pectoris (5, 6) and in bronchial asthma (7) prompted the Cardiovascular Department of this institution to investigate the effect of khellin in these diseases and in chronic cor pulmonale. The results of these studies have been previously reported elsewhere (8). In that study eight patients, clinically judged to have chronic cor pulmonale, were included. This group was characterized by pulmonary disease, diminished vital capacity, and in some instances, clubbing of the fingers and cyanosis. Four of the patients were in congestive heart failure. In all eight patients, khellin therapy resulted in improvement characterized by amelioration of dyspnea and wheezing, decreased cyanosis, increase in exercise capacity and an increased sense of well being.

In order to delineate the mechanism responsible for the improvement noted in these patients, and to apply objective physiological criteria to such studies, the present cardio-pulmonary investigation was undertaken.

METHOD

The patients in the present study consisted of six males with chronic pulmonary disease, not all of whom were as ill as those in the previous study (8). The patients were out-patients selected from the Mandel Clinic. Physical examination, fluoroscopy, chest films and electrocardiograms were taken shortly before the study. The electrocardiogram was normal in one patient and abnormal in five, but none showed evidence of right heart strain. The hemoglobin content of the blood ranged from 13.7 to 20.8 gm.%; the hematocrits, from 48 to 59. The clinical data are summarized in Table I. All therapy was stopped for four to seven days before the study.

The general plan of the study was to determine the effect of khellin on certain aspects of the respiratory and circulatory dynamics. The technique of right heart catheterization was combined with the simultaneous determination of respiratory gases in the arterial blood and in expired air, at rest and during exercise. Studies were made immediately before and after an intramuscular dose of the drug and were repeated after five to eight days of daily oral administration.

Preliminary respiratory studies, consisting of maximum breathing capacity, vital capacity, inspiratory capacity and expiratory reserve volume (9) were determined at the beginning of the study. For this purpose a 9 liter Benedict-Roth spirometer was used. It was fitted with a vitalometer and the flutter valves and the soda lime chamber were removed (10). On the day of catheterization, the patient came to the cardio-pulmonary laboratory in the post-absorptive state. The cardiac catheter was introduced into the pulmonary artery or one of its main branches according to the technique of Cournand and Ranges (11). An indwelling needle was inserted into the brachial artery for the duration of the procedure.

Expired air was collected using an open circuit technique. A face mask was connected through a Robinson type, high-velocity valve to three Douglas bags and a Tissot spirometer in series. After an initial period of recumbency of at least 20 minutes, a five minute resting sample of expired air was collected, and, half way through this period, simultaneous brachial artery and pulmonary artery blood samples were obtained for the determination of resting cardiac output. Resting pulmonary and brachial arterial pressures were recorded by means of a capacitance

[1] Aided in part by a grant from the National Heart Institute (H 218) and a grant from Smith, Kline and French Laboratories.

[2] Khellin was supplied in the form of Eskel by Smith, Kline and French Laboratories.

[3] Postdoctorate Research Fellow, National Heart Institute.

[4] These departments are aided in part by the Michael Reese Research Foundation.

TABLE I

Clinical data on patients studied

Case	Age	Surface area	Symptoms				Physical findings				Roentgenographic findings	Diagnosis
			Dyspnea	Chronic cough	Expectoration	Other	Cyanosis	Emphysema	Rales	Cong. failure		
		M^2										
1	61	1.84	Exertional, 7 yrs. At rest, 2 yrs.	+	+	Recurrent broncho-pulmonary infections	+	+	Persistent basal	0	Diaphragms flat and immobile. Increased bronchovascular markings. Prominent pulmonary arteries	Chronic hypertrophic emphysema. Bronchiectasis. Chronic pneumonitis, right ower lobe. Pulmonary calcification
2	65	2.06	Progressive, 1943–1950. Incapacitating since 1947	+	0	Emory wheel grinder—1942–1945. Symptoms worse with respiratory infection	0	+	Inconstant at both bases	0	Diffuse, fine, reticular and nodular infiltration bilaterally. Increased bronchovascular markings, right lower lobe. No hilar lymph adenopathy	Silicosis. Chronic bronchial infection
3	59	1.95	Exertional, 1 yr.	0	0	Bituminous coal miner, many yrs. Steel mill worker	0	0	Bilateral coarse	0	Diffuse reticular infiltration in lower 2/3, both lung fields	Anthracosis
4	62	1.64	Progressive, 10 yrs. Incapacitating, 1 yr.	+	+	Slow weight loss. Wheezing	0	+	Bilateral basal	0	Increased bronchovascular markings. Prominent pulmonary artery trunk. Diaphragms flat and immobile	Pulmonary emphysema. Pulmonary fibrosis
5	74	1.70	Exertional	+ (25 yrs.)	+	Wheezing. Repeated pulmonary infections	0	+	Bilateral basal	0	Linear fibrosis, both bases. Pleural scarring right base. Aorta widened. Heart upper limits of normal size	Bilateral basal bronchiectasis
6	73	1.67	Progressive, 17 yrs.	0	0	Bituminous coal miner, 20 yrs.	0	+	Left base	0	Diffuse reticular and nodular infiltration. No hilar adenopathy. Pulmonary arteries prominent. Pleural scarring, right with fixation of diaphragms	Anthrasilicosis? Diffuse pulmonary fibrosis

electromanometer and direct writing multi-channel oscillograph.[5] The patients then exercised in the recumbent position, using a bicycle fitted with a brake and attached to the end of the fluoroscopic table. The exercise period was three minutes except in case 2 where dyspnea supervened at 2½ minutes, and in case 6 where the patient developed pain in the right hip after two minutes. The exercise was only roughly standardized. The expired air was collected separately in three Douglas bags for (*1*) the first minute of exercise, (*2*) the remainder of the exercise period, and (*3*) the first minute of recovery. The expired air from the beginning of the second minute of recovery through the fifth minute of recovery was collected in the Tissot spirometer. The volumes of gas in the Douglas bags and the Tissot were determined, and samples were taken for analysis.

After a rest period of 20 minutes, the resting determinations were repeated and the patient was given 300 mg. of khellin intramuscularly. Twenty minutes later the resting and exercise determinations were again carried out. After completion of these studies the patient was maintained in the hospital on unrestricted activity and was given 200 mg. of khellin orally for four to seven days. Except for penicillin given for two days after catheterization, as prophylaxis against infection, the patient received no other medication. At the end of this period of time, the cardio-pulmonary studies were repeated at rest and during exercise. The maximum breathing capacity, vital capacity and its compartments were repeated after the second catheterization.

Blood gas analysis was carried out according to the technique of Van Slyke and Neill (12). The expired air samples were analyzed for oxygen by a Pauling oxygen analyzer, and for carbon dioxide by the Haldane technique. The average minute ventilation for the various periods was calculated and expressed as liters per minute at 37° C. ambient pressure and saturated with water vapor (B.T.P.S.). The oxygen removal in cc. per liter of ventilation, the average oxygen uptake in cc. per minute (10), and the oxygen debt for the entire five minutes of recovery were also calculated. The oxygen debt was calculated as follows:

$$\frac{O_2 \text{ uptake 5 min. recovery} - O_2 \text{ uptake 5 min. rest}}{(O_2 \text{ uptake 3 min. exercise} + O_2 \text{ uptake 5 min. recovery}) - O_2 \text{ uptake 8 min. rest.}} \times 100 = O_2 \text{ debt } \%.$$

Cardiac output was determined by the direct Fick principle.

RESULTS

On initial study the resting arterio-venous oxygen difference and cardiac index were within normal limits in all cases. The cardiac indices ranged from 2.0 to 3.7 L/min./M²B.S.; the A–V differences, from 4.1 to 5.9 vol.%. The pulmonary arterial pressure was elevated in two cases, one of which (case 1) had an elevation of the diastolic pressure as well. All of the patients demonstrated a resting oxygen uptake which was within normal limits, except case 5 in which the oxygen uptake was elevated at rest initially, but fell to a normal level 25 minutes after exercise. The ventilation at rest was elevated in all patients except case 4. The oxygen removal was decreased in all cases. Thus all patients demonstrated some degree of hyperventilation at rest (Table II).

The response to exercise was normal in all cases with respect to cardiac index and arterio-venous oxygen difference (13). The cardiac indices ranged from 3.4 to 5.1 L/min./M²B.S. representing 140–250% of the resting values. The arterio-venous oxygen differences ranged between 6.7 to 8.2 vol.% representing 127–187% of the resting values. During exercise, the oxygen uptake rose to between two and 3⅓ times the resting level (Table II). Hyperventilation persisted during exercise, but there was a rise in the oxygen removal above the resting level. This change was demonstrated in all cases. The oxygen debt for the first five minutes of recovery ranged between 13% and 22% in the four cases in which this information was available. Exercise produced a rise in systolic and diastolic pulmonary arterial pressure in five cases. In one case, there was elevation only of the systolic pressure. The greatest increment of pressure rise occurred during systole, ranging from 10 to 30 mm. Hg. The diastolic pressure rise varied from 4 to 10 mm. Hg. In two cases (cases 5 and 6) pulmonary artery and right ventricular pressures were recorded simultaneously. In case 6, the diastolic pressure in the right ventricle rose to abnormal levels during exercise, indicating acute right heart failure (Table II).

Twenty-five minutes after the completion of exercise, and just prior to the injection of khellin, the cardiac index was at or below the previous resting level in three cases. In the remaining cases, the cardiac index remained above the original resting level. The arterio-venous difference was essentially unchanged in five cases. In one case, the arterio-venous difference was below resting level. The oxygen uptake deviated from the initial determination by 20 cc./min./M²B.S. or less in four cases. In one case the oxygen uptake was

[5] Sanborn polyviso.

TABLE II

Summary of data obtained during catheterization

Case	Catheterization	Relation to khellin administration	State	Ventilation, *L/min./M²B.S.* B.T.P.S.	Oxygen uptake, *cc./min./M²B.S.* S.T.P.D.	Oxygen removal, *cc./L ventilation*	Oxygen debt (partial) 1st 5 min. recovery (%)	Ratio: oxygen uptake exer. / oxygen uptake rest	Arteriovenous oxygen difference, *vol. %*	Cardiac output *L/min.*	Cardiac index *L/min./B.S.*	Pressure	
												Pulmonary artery, *mm. Hg*	Right ventricle, *mm. Hg*
1	1st	Control	Rest	5.8	146	31.4	—	—	4.3	6.3	3.4	35/15(24)†	
			Exercise	—	—	—			6.7	—	—	50/19(34)	
			Rest*	8.0	160	32.7			4.0	9.7	5.3	40/15(22)	
		After 300 mg. khellin intramuscularly	Rest	6.2	160	32.4	—	—	3.5	8.5	4.6	35/12(19)	
			Exercise	—	—	—			—	—	—	35/15(20)	
	2nd	After 9 days of 200 mg. khellin per day orally	Rest	5.3	144	33.5	—	—	4.2	6.3	3.4	37/12(28)	
			Exercise	—	—	—			5.8	—	—	56/18(38)	
2	1st	Control	Rest	5.8	12.0	25.9	13	2.3	5.2	4.8	2.3	20/10(15)	
			Exercise	10.8	27.5	31.8			7.7	7.5	3.6	50/17(30)	
			Rest*	6.7	137	25.5			4.4	6.5	3.2	30/10(20)	
		After 300 mg. khellin intramuscularly	Rest	6.1	129	26.4	17	2.1	4.5	6.0	2.9	27/10(17)	
			Exercise	9.9	268	33.9			6.3	8.8	4.3	47/17(30)	
	2nd	After 8 days of 200 mg. khellin per day orally	Rest	4.9	95	23.9	—	—	5.4	3.7	1.8	20/15(20)	
			Exercise	—	—	—			8.4	—	—	60/20(35)	
3	1st	Control	Rest	5.0	124	30.6	20	3.3	5.9	3.9	2.0	22/7(15)	
			Exercise	13.7	405	36.9			7.5	10.0	5.1	35/7	
			Rest*	10.0	167	20.7			3.4	9.1	4.7	20/7	
		After 300 mg. khellin intramuscularly	Rest	7.8	145	23.1	7	2.1	4.1	6.5	3.3	22/7	
			Exercise	10.2	303	36.9			6.9	8.1	4.2	40/10	
	2nd	After 5 days of 200 mg. khellin per day orally	Rest	8.9	154	24.4	20	4.0	5.3	5.4	2.8	20/5(10)	
			Exercise	19.4	617	39.2			10.8	10.6	5.4	40/5	

TABLE II—*Continued*

Case	Catheterization	Relation to khellin administration	State	Ventilation, *L/min./M²B.S.* B.T.P.S.	Oxygen uptake, *cc./min./M²B.S.* S.T.P.D.	Oxygen removal, *cc./L ventilation*	Oxygen debt (partial) 1st 5 min. recovery (%)	Ratio: oxygen uptake exer. / oxygen uptake rest	Arterio-venous oxygen difference, *vol. %*	Cardiac output *L/min.*	Cardiac index *L/min./B.S.*	Pressure	
												Pulmonary artery, *mm. Hg*	Right ventricle, *mm. Hg*
4	1st	Control	Rest	4.7	118	30.9	14	2.2	4.1	4.4	2.7	18/2(12)	
			Exercise	9.8	258	36.7			7.7	5.5	3.4	28/10	
			Rest*	4.6	124	33.8			4.4	4.7	2.9	18/8(12)	
		After 300 mg. khellin intramuscularly	Rest	5.0	128	31.9	—	2.0	4.1	5.2	3.2	18/8(12)	
			Exercise	9.6	258	33.2			7.0	6.1	3.7	35/12	
	2nd	After 5 days of 200 mg. khellin per day orally	Rest	6.3	155	30.4	24	2.6	5.3	4.8	2.8	20/7(10)	
			Exercise	12.9	396	38.2			8.4	7.8	4.8	30/18	
5	1st	Control	Rest	9.1	183	25.0	22	2.0	5.0	6.3	3.7	24/6(8)	15/2‡
			Exercise	13.1	374	28.6			7.3	8.9	5.2	48/16	40/6
			Rest*	6.5	146	28.1			5.1	4.9	2.9	30/8(10)	18/0‡
		After 300 mg. khellin intramuscularly	Rest	5.0	134	33.2	20	2.6	4.3	5.4	3.2	28/10	24/0
			Exercise	12.8	348	43.7			7.3	8.3	4.9	50/18(22)	40/4
6	1st	Control	Rest	7.0	147	26.4	—	2.2	5.3	4.6	2.8	38/10(20)‡	60/2(13)
			Exercise	18.6	322	21.8			8.2	6.6	4.0	59/20(35)‡	91/10(34)
			Rest*	6.8	127	23.5			5.6	3.8	2.3	50/19(20)	52/10(20)
		After 300 mg. khellin intramuscularly	Rest	7.2	142	24.7	—	2.5	4.9	4.8	2.9	50/17(30)	56/4(20)
			Exercise	19.0	351	23.2			7.8	7.5	4.5	99/38	100/10

Exercise determinations were made during the last two minutes of a three minute exercise period except in case 2 where duration of exercise was only 2½ minutes and case 6 where it was two minutes. In these two cases collections were made during the last 1½ and one minute of exercise, respectively.

* Resting determination 25 minutes after end of exercise.
† Numbers in parentheses, mean pressures.
‡ Damped curves.

significantly higher and in another was significantly lower than the initial determination. In general, ventilation showed similar changes. In four cases the pulmonary artery pressure returned to the original resting levels. In one case, the systolic pressure remained elevated (case 2). In one case, the character of the pulmonary artery curves did not permit adequate comparison. However, comparison of ventricular pressure curves during the resting states shows systolic levels of the same order of magnitude (Table II).

The arterial blood oxygen saturation was within normal limits at rest in three cases, but was below 95% in cases 4 and 6. With exercise, there was a fall in the arterial oxygen saturation in the four cases in which this was measured. Only in case 3 did the arterial oxygen saturation, during exercise, remain within normal limits. The resting carbon dioxide contents were below normal limits in all except case 4. The response to exercise consisted of a slight rise in the CO_2 content in two cases and a slight fall in three (Table III).

Twenty minutes after the injection of 300 mg. khellin, there was no significant change from the resting values of the arterio-venous oxygen difference, cardiac index, and pulmonary arterial pressure (Table II). In four of the five cases for which such data are available, the changes in arterio-venous oxygen difference and cardiac index rose to the same extent with exercise as was observed prior to administration of the drug. In one case (case 3) the cardiac index rose less with exercise than before khellin. The resting oxygen uptake, ventilation and oxygen removal showed no consistent deviation from the resting data obtained 25 minutes following exercise. In case 3 the oxygen uptake was considerably higher and in case 5 was considerably lower than the original determination. During exercise these values were remarkably similar to those obtained during the control period except in case 3 where the oxygen uptake was lower and case 5 where the oxygen removal was higher. Five of the six cases showed a rise in the pulmonary artery pressure to the same degree as before khellin administration. One case (case 1) showed no change in pulmonary artery pressure (Table II).

A second catheterization was performed in four cases following several days of administration of oral khellin. At rest the arterio-venous oxygen difference, cardiac index and pulmonary arterial pressures were unchanged from the resting level obtained during the control period of the first catheterization. During exercise the increase in arterio-venous oxygen difference was of the same order of magnitude as that observed with exercise during the control period of the first catheterization in three cases, while in one case (case 3) it was significantly increased. The cardiac index in the two cases in which cardiac output was measured was increased to the same extent as during the control period of exercise. No significant changes in oxygen uptake, ventilation or oxygen removel were noted during rest or exercise. In case 3, the severity of exercise was greater than during the control period but there was no significant increase in oxygen removal. The oxygen debt data are incomplete, and the changes noted are probably not significant. Bruce and his associates (14) and Baldwin, Cournand, and Richards (10)

TABLE III

Summary of blood gas data

Arterial blood gases	Case 1	Case 2		Case 3		Case 4		Case 5	Case 6
	Before khellin	Before khellin	After 8 days of khellin	Before khellin	After 5 days of khellin	Before khellin	After 7 days of khellin	Before khellin	Before khellin
Oxygen Saturation, %									
Resting	80.5	95.5	94.5	100	95	86.6	88.2	97.2	87.2
Exercise	—	—	91.5	93	88.6	77.0	81.0	86.2	79.7
CO_2 Saturation, %									
Resting	—	45.1	45.8	38.8	39.9	53.3	50.9	42.4	44.3
Exercise	—	42.9	—	41.0	41.9	56.4	53.4	41.0	43.2

TABLE IV

Summary of respiratory data

Case	Time	Maximal breathing capacity in liters		Vital capacity in cc.		Inspiratory capacity in cc.		Expiratory reserve volume in cc.	
		Determined	Predicted	Determined	Predicted	Determined	Predicted	Determined	Predicted
2	Pre-khellin	81	110	2,730	3,560	800	640	1,900	2,920
	After 5 days on khellin	102	—	3,300	—	1,440	—	1,900	—
3	Pre-khellin	97	109	2,990	3,510	500	630	2,200	2,910
	After 5 days on khellin	110	—	3,030	—	900	—	2,260	—
4	Pre-khellin	25	89	2,270	3,460	660	600	1,720	2,860
	After 6 days on khellin	31	—	3,070	—	1,270	—	2,220	—
5	Pre-khellin	52	82	2,550	2,990	860	520	1,740	2,470
	After 8 days on khellin	72	—	2,300	—	910	—	1,560	—
6	Pre-khellin	65	81	2,280	3,340	950	580	1,590	2,760

have pointed out the great variability of this determination. The response of the pulmonary arterial pressure to exercise in all four cases was similar quantitatively to that observed prior to administration of the drug (Table II).

All of the spirographic tracings showed trapping of air. The maximum breathing capacities were reduced in all cases, ranging from 36% to 89% of the predicted values. The vital capacities were also reduced but to a lesser degree than that of the maximum breathing capacities (Table IV). The spirographic tracings showed slowing of expiration in all cases, marked in cases 2, 4 and 5. The maximum breathing capacity was performed in an extreme inspiratory position in all instances, except case 3. This patient performed the test in the mid-position and showed the least reduction from the predicted value. In four patients spirometry was repeated after a number of days of administration of 200 mg. of khellin daily. These patients displayed a slight to moderate increase in the maximum breathing capacity; the mean increase was 15 L/min., representing changes of 24%, 27%, 39% and 14% from the control level. The vital capacity was significantly increased in two cases with no significant change in the others. The expiratory reserve volume was increased in one case, decreased in one case and unchanged in two.

There were no objective or subjective clinical signs of improvement noted after the intramuscular injection of khellin.

At some time during the course of oral khellin administration, all of the patients volunteered the information that it was "easier to get air in and out of the chest." There was no objective improvement in the cyanosis, dyspnea, exercise capacity, or physical findings.

DISCUSSION

The six patients comprising this study were selected on the basis of extensive chronic pulmonary disease and clinical disability which led to the suspicion of chronic cor pulmonale. Neither roentgenographic evidence of right ventricular enlargement nor electrocardiographic evidence of right heart strain was present in these cases. It is well known, however, that chronic cor pulmonale may exist in the absence of these findings. The unreliability of these diagnostic aids and of the clinical symptomatology has made the diagnosis of chronic cor pulmonale uncertain in the absence of right heart failure or a previous history of such an episode. This has been previously pointed out by Ferrer and his associates (15).

Only two of the six cases displayed pulmonary hypertension at rest; the remaining four developed pulmonary hypertension during exercise. In one of the patients with resting hypertension, pressures were recorded from the right ventricle and exhibited elevation of the end diastolic pressure in this chamber to early failure levels during exercise. Thus, it can be seen that only one of our patients

may be considered to have proven cor pulmonale, the remaining five are examples of pulmonary hypertensive heart disease without proven cor pulmonale.[6]

The cardiac output at rest and with exercise was normal in this group of patients. Impaired respiratory function, varying from mild to severe, was demonstrated by the maximum breathing capacity and vital capacity. In addition, hypoxemia was present to some degree in three of the six cases and developed in the other three during exercise. All of the patients demonstrated hyperventilation both at rest and during exercise, but showed a rise in oxygen removal during exercise. Since the oxygen uptake was within normal limits the hyperventilation may be considered compensatory.

Khellin administered intramuscularly produced no significant changes in the cardiac output. There was no alteration of the pulmonary arterial pressure at rest, nor was there any demonstrable protective action in preventing a rise in the pulmonary arterial pressure with exercise. In this regard, in case 1, exercise after khellin was accompanied by no rise in pulmonary arterial pressure. However, during the second catheterization following intramuscular khellin, the pulmonary pressure rose in the same manner observed in all other patients. Since measurements of cardiac output during exercise were not obtained in this case and the exercise was not precisely quantitated, the failure of the pulmonary artery pressure to rise at the time of the first catheterization cannot be accurately appraised. The occurrence of pulmonary hypertension with exercise during the second catheterization would suggest the first observation in this case has no significance.

Intramuscular khellin produced no consistent changes in ventilation, oxygen uptake and oxygen removal. The changes which occurred were probably within the limits of the experimental error.

Prolonged administration of khellin did not alter the hemodynamic pattern observed before its administration. Cardiac output and pulmonary pressure at rest and their responses to exercise were essentially unchanged. However, there was an increase in maximum breathing capacity in the four cases in which this information was available. No constant changes were found in ventilation, oxygen uptake, oxygen removal or oxygen debt. Khellin did not appear to have any "protective" effect on the arterial saturation during exercise.

Correlation of the subjective clinical improvement with the increase in maximum breathing capacity without any change in the other cardiorespiratory functions studied, suggests that khellin had a bronchodilator effect in these four cases. All of the patients were judged to be clinically free of active bronchopulmonary infection at the time of study and for this reason it was felt that the effect of brief administration of penicillin on the aspects of pulmonary function measured could be disregarded. Nevertheless, the possibility that the observed increase in maximum breathing capacity was, at least in part, on this basis cannot be excluded. Study of a larger series of cases is necessary to properly evaluate the significance of this observation. The increase in maximum breathing capacity was not accompanied by any diminution of pulmonary arterial pressure; the effect of more potent bronchodilators deserves investigation in this regard.

It is apparent that there is a striking disparity in the results previously reported by Rosenman and his associates (8) and the results obtained in this study. This discrepancy may be due in part to the apparently greater severity of disability of the patients in the earlier study and in part to the well known difficulty in accurately evaluating improvement to any method of therapy by non-objective criteria.

SUMMARY

1. Six patients with chronic pulmonary disease were studied with respect to the effect of khellin on certain aspects of cardio-pulmonary function.

2. Khellin given intramuscularly produced no consistent change during rest or exercise in ventilation, oxygen uptake, oxygen removal, cardiac output or pulmonary arterial pressure. The changes which did occur were probably within the limits of the experimental error.

3. Restudy after a course of oral khellin, revealed an increase in maximum breathing capacity in the four cases in which this information was available. No consistent changes were found in ventilation, oxygen uptake, oxygen removal,

[6] Pulmonary hypertensive heart disease is the subject of a forthcoming publication.

cardiac output or pulmonary arterial pressure. Khellin did not appear to have any "protective" effect on the arterial saturation during exercise.

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EFFECTS OF EXOGENOUS AND ENDOGENOUS POSTERIOR PITUITARY ANTIDIURETIC HORMONE ON WATER AND ELECTROLYTE EXCRETION [1]

By RICHARD J. F. MURPHY [2] and EUGENE A. STEAD, JR.

(From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

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The urine output of normal subjects varies greatly from hour to hour. Two of the important factors affecting the urine volume are the degree of activity of the posterior pituitary gland and the amount of solids to be excreted in the urine. In general, whenever the normal kidney is excreting a concentrated urine of low volume, secretion of the antidiuretic hormone (ADH) of the posterior pituitary gland is occurring. This secretion may be due to emotional, drug, or other nervous stimulation, or to an increased osmotic pressure in the extracellular fluid. The excretion of a large amount of very dilute urine by the normal kidney occurs when ADH production is inhibited either by nervous or osmotic stimuli. When concentrated electrolyte solutions are administered intravenously, a large quantity of concentrated urine is produced. This large urine volume appears to be the result of the osmotic effects of the solids in the urine, and the volume is not decreased by secretion of ADH.

While there is general agreement as to the effects of ADH on water excretion, there has been little information on its effects on sodium, chloride and potassium excretion in man. The purposes of this study were (*1*) to determine the effect of endogenously produced ADH on the excretion of water, sodium and chloride, (*2*) to determine whether minute doses of commercial Pitressin given intravenously had the same effects on water and electrolyte excretion as endogenously produced ADH, (*3*) to determine whether the rate of excretion of sodium modified the antidiuretic effectiveness of Pitressin, and (*4*) to determine whether Pitressin given intravenously in minute doses altered glomerular filtration rate or renal plasma flow.

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[2] Present Address: Department of Medicine, Royal Victoria Hospital, Montreal, Canada.

MATERIALS AND METHODS

All of the following studies have been carried out on human subjects without endocrine, renal, cardiac, or other disease which has a known effect on water and electrolyte metabolism. All had been on normal salt and water intakes in the past. All subjects were studied in the post-absorptive state as regards food. The differing water and electrolyte conditions which were introduced as a part of the study will be mentioned specifically in the results.

In all studies the same lot of Pitressin (commercial ADH prepared by Parke, Davis and Company) has been used. Fresh solutions of Pitressin in .9% NaCl were prepared for each experiment. The doses varied somewhat as specifically mentioned but where it is not mentioned, the dose was arbitrarily fixed at 1.0 milliunit per kilo of body weight.

Urine collections were made from an indwelling urethral catheter which was placed at the bladder neck with considerable care. Throughout the full period of each study, which was carried out in a quiet, isolated environment, the patient was recumbent but not sleeping. As much assurance as possible was given to avoid any emotional upset during the simple procedures that were carried out.

In all cases except the clearance studies, urine was collected at arbitrary time intervals of 10 minutes. Individual samples were measured for chloride by the Van Slyke and Hiller modification of the method of Sendroy (1). Sodium and potassium were measured with the Perkin-Elmer flame photometer.

In measuring the renal hemodynamic effect of Pitressin, the clearance techniques of Smith, Goldring, and Chasis have been followed using inulin and sodium paraminohippurate (PAH) as the test substances (2).

RESULTS

Endogenous secretion of pituitary ADH. After collecting urine for 20–30 minutes, sodium chloride solution in doses of 20–30 cc. per kilo of body weight was given intravenously over a period of 30–60 minutes to 21 normal subjects. These individuals may be divided into two groups, depending on the concentration of the salt used. Group I consisted of 15 subjects who received a solution with a concentration of 125–146 millimols of sodium chloride; Group II of six subjects who re-

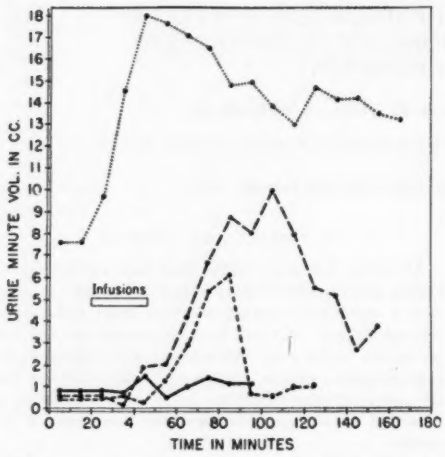

FIG. 1. EFFECT OF NaCl INFUSIONS ON URINE VOLUME IN THREE NORMAL SUBJECTS AND ONE PATIENT WITH DIABETES INSIPIDUS

The findings in normal subjects are shown in the three lower curves. The subjects receiving NaCl solution of 155 millimols are represented by the continuous line; that of 142 millimols by short dashes; and that of 125 millimols by long dashes. The concentration of the NaCl solution in the patient with diabetes insipidus was 139 millimols (dotted line).

ceived a solution with a concentration of 149–155 millimols. It is generally stated that .9% sodium chloride solution (155 millimols) is isotonic with plasma.

In Group I the initial effect was the production of a water diuresis (i.e., the urine minute volumes increased markedly to levels of 6–12 cc./min. and sodium and chloride concentrations decreased) beginning about 30 minutes after the infusion was started. After a variable but short period of time there was an abrupt antidiuresis. As shown in Figure 1, the more concentrated solutions in Group I produced an earlier antidiuresis. In Group II, who received saline in slightly greater concentration, no water diuresis was produced. The urine volume increased slowly over the period of observation but it did not rise above 3 cc./min., and there was no abrupt antidiuresis. As the urine volume increased in this group the sodium concentration in the urine rose and did not decrease as it did in Group I. In Figure 1 the effect of giving 1,400 cc. of sodium chloride solution with a concentration of 139 millimols to a patient with diabetes insipidus is shown. There was an immediate large increase in urine volume with a slow fall over the next two hours. There was no abrupt antidiuresis as there was in Group I.

The following interpretation of these data is offered: Solutions containing a concentration of between 125 and 146 millimols of sodium chloride do not stimulate the production of ADH. Their initial effect is to cause a water diuresis either by dilution of existing ADH or by inhibition of its secretion. During water diuresis the loss of water greatly exceeds the loss of salt and the electrolytes in the body are concentrated. In due time this causes an osmotic stimulus for ADH secretion and antidiuresis occurs. The absence of a water diuresis when the concentration of sodium chloride is slightly increased in the fluid administered suggests that the osmotic mechanism is a sensitive one and that the amount of water lost does not have to be large to cause the production of endogenous ADH. As the concentration of the NaCl solution approaches 150 millimols, the duration of the water diuresis is progressively reduced (Figure 1). The fact that the patient with diabetes insipidus

TABLE I

Transition from diuresis to antidiuresis following infusion of NaCl solution of concentration 125–146 mM. Lack of effect on excretion of sodium and chloride

Subj.	Water excretion (*cc./min.*)							Chloride excretion (*meq./10 min.*)							Sodium excretion (*meq./10 min.*)						
D. A.	10.8	9.0	8.1	7.2	3.8	2.8	3.5	7.0	7.0	6.6	6.5	5.1	4.3	5.3	7.8	6.9	5.8	6.4	5.3	4.4	5.4
W. M.	7.5	12.6	12.8	8.5	4.5	3.5	2.9	4.5	7.1	6.3	5.4	6.2	5.9	5.4	3.4	6.1	5.5	4.4	5.6	5.4	4.7
H. C.	4.0	5.6	4.7	4.5	2.9	2.4	2.4	1.6	1.8	1.7	2.1	2.7	2.5	3.0	2.2	2.2	2.0	2.4	3.2	3.1	3.1
E. T.	15.7	13.5	9.4	8.7	8.0	5.5	5.8	4.4	4.4	4.2	4.5	4.6	3.6	4.8	5.4	5.5	4.8	5.4	5.7	4.3	5.9
G. H.	12.5	15.4	8.2	4.5	4.6	5.2	5.4	6.8	7.1	6.9	6.6	7.6	7.8	7.9	7.4	6.9	7.3	7.4	8.4	9.7	8.3
E. A.	14.0	13.4	11.8	9.5	6.2	5.1	3.9	4.5	4.6	4.6	4.5	4.2	4.3	4.3	3.2	3.0	3.1	3.0	2.9	2.6	2.9
A. M.	13.2	13.3	14.1	10.7	4.2	3.2	3.5	5.3	4.5	4.9	5.0	4.2	4.8	5.1	5.7	4.9	5.2	6.0	4.6	5.9	6.3
E. T.	9.8	8.5	6.3	3.7	2.6	2.7	2.6	2.3	2.0	1.9	1.7	1.7	1.8	1.9	2.0	1.6	1.8	1.8	1.8	1.9	2.0

TABLE II

Effect of small, intravenous doses of Pitressin on urine volume and sodium and chloride excretion in the absence of endogenous secretion of ADH

Observations were made during infusion of PAH and insulin

Subj.	Urine volume (*cc./min.*)				Urine chloride (*μeq./min.*)				Urine sodium (*μeq./min.*)			
	Before Pitressin		After Pitressin		Before Pitressin		After Pitressin		Before Pitressin		After Pitressin	
	Per. I	Per. II	Per. I	Per. II	Per. I	Per. II	Per. I	Per. II	Per. I	Per. II	Per. I	Per. II
D. W.	9.6	12.0	1.9	2.2	73	70	82	62	119	89	133	107
M. B.	9.6	10.1	2.0	2.1	135	154	250	290	133	157	225	308
L. C.	6.8	8.3	0.2	0.8	42	53	49	64	41	69	49	79
E. M.	10.2	12.2	0.3	0.8	80	80	58	57	94	94	68	66
L. H.	14.6	14.9	0.8	1.3	185	162	123	110	257	221	139	143
R. T.	12.3	13.8	1.1	0.4	123	151	123	90	143	171	143	89
M. C.	17.1	17.7	0.3	1.4	322	296	218	254	411	389	241	291
H. R.	8.2	6.2	2.3	2.5	309	277	305	235	366	336	391	324
R. T.	10.9	11.5	1.0	1.0	141	139	144	119	128	138	117	103

did not have the abrupt antidiuresis which the normal subjects had supports the above interpretation. O'Connor (3) has shown that in the dog changes between a fall of 2.5% and an increase of 5% from the resting level of blood chloride were sufficient to cause the tubules to respond over the full range of their functional capacity from maximal selective reabsorption of water when salt was given to minimal reabsorption in water diuresis.

Granting that the antidiuresis produced when NaCl solutions between 125 and 146 millimols are given intravenously results from the secretion of endogenous ADH, we are in a position to determine the effect of this material on electrolyte excretion. Table I shows these results in eight normal subjects in whom complete data on sodium and chloride excretion are available. Despite a change in urine volume from a mean of 11 cc./min. to 3.8 cc./min. the excretion of sodium and chloride remained constant. Under the conditions of these observations endogenous ADH neither increases nor decreases sodium and chloride excretion.

EFFECT OF MINUTE DOSES OF PITRESSIN ON THE EXCRETION OF WATER AND ELECTROLYTES

Shannon has shown that the full range of antidiuretic effects can be produced in the intact dogs by the intravenous administration of 1–5 milliunits of Pitressin per hour (4). Lauson and his colleagues reported identical findings in man with allowance for weight differences (5). In the dog (6) and presumably in man, much greater quantities of ADH are produced in response to certain physiologic stimuli than are needed for maximal antidiuretic activity. In five normal subjects the endogenous secretion of ADH was reduced to a minimum by oral administration of 1,000–2,000 cc. of water and the antidiuretic effects of giving a single intravenous injection of 5 to 70 milliunits of Pitressin were observed (Figure 2). If one allows five minutes as an average figure for the passage of urine from the renal tubules to the bladder, it appears that the antidiuretic effect is produced immediately. It is also apparent that over a fairly wide range of this drug, the duration of maximal antidiuresis is relatively fixed. In

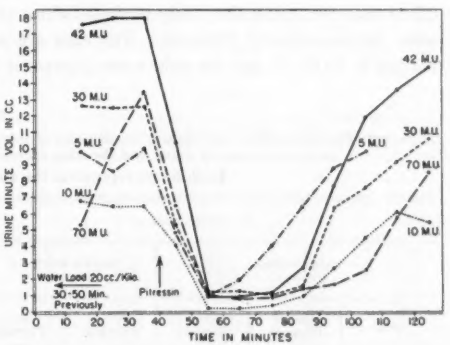

FIG. 2. EFFECT OF SMALL VARYING DOSES OF PITRESSIN ON THE COURSE OF WATER DIURESIS IN NORMAL SUBJECTS

Each point represents the average minute volume of a 10 minute collection period and is placed at the five minute point in the period.

four of these five subjects receiving doses varying from 10–70 milliunits, maximal antidiuresis lasted about 40 minutes and an additional 30–40 minutes were required for the tubules to "escape" from the antidiuretic effect. In the one subject receiving only 5 milliunits, onset of maximal antidiuresis was similar to the other individuals but the duration of maximal antidiuresis was shorter and the "escape" from its effect more immediate.

Doses of 0.5 to 2 milliunits of Pitressin per kilo of body weight given intravenously over a two minute period of time produced no detectable effect on pulse rate, blood pressure, skin color, or gastrointestinal activity.

The effect of Pitressin on electrolyte excretion was measured under three differing circumstances. The first was in conjunction with the study of renal blood flow and glomerular filtration rate. These subjects were previously water-loaded to suppress endogenous secretion of ADH. It has been stated that in dogs, this is the only way to demonstrate the "chloruretic" properties of this preparation (7). All these subjects were given a standard volume of water to drink (20 cc. per kilo of body weight) 30–50 minutes before the onset of the studies so that they had a good water diuresis by the time the pre-Pitressin collections were made. These measurements were made during the infusion of inulin and PAH. Hippurate, being an acid salt which is rapidly cleared by the tubules, has an effect on the excretion of sodium. This effect was present in the study periods before and after the injection of Pitressin. The data are recorded in Table II and the values are expressed in terms of excretion per minute because the time of the individual periods ranged from 13–18 minutes. Despite the striking change in urine volume from 11.0 ± 1.1 cc. and 11.9 ± 1.1 cc. in the two periods before Pitressin to 1.1 ± 0.3 cc. and 1.4 ± 0.2 cc. in the two periods after Pitressin the excretion of sodium and chloride was not significantly changed. Potassium excretion was not measured in this group.

The second group of seven subjects received a water load of 20 cc. per kilo of body weight. They differed from the first group in that they had had no injections of inulin or hippurate. A 30 minute collection of urine was made before and after the injection of Pitressin. The urine was not collected during the first 10 minutes after the injection of Pitressin. In this group there was a slight but constant decrease in the excretion of sodium. The excretion of chloride in those cases where it was measured followed sodium. Potassium excretion was not measured. The data are recorded in Table III.

The third group of eight subjects received 1,000–1,500 cc. of 0.8–0.9% NaCl solution intravenously before urine collections were started. The infusion was finished one to two hours before Pitressin was given. This insured a relatively high concentration of sodium and chloride in the urine. These patients undoubtedly had some endogenous ADH effect because the urine volume was considerably smaller than in the previous study. They received no inulin or hippurate. In this group, urine collections were made at 10 minute intervals to note any pattern responses. Figure 3 shows the effect

TABLE III

Differing effects on sodium excretion of small doses of intravenous Pitressin in two groups, one excreting small amount of salt in large volume of urine and the other excreting larger amount of salt in a smaller volume of urine

Each figure represents the average of a 30 minute collection period

A. Water load					B. Saline load				
	Urine volume (*cc./min.*)		Sodium excretion (*meq./min.*)			Urine volume (*cc./min.*)		Sodium excretion (*meq./min.*)	
Subj.	Before Pitressin	After Pitressin	Before Pitressin	After Pitressin	Subj.	Before Pitressin	After Pitressin	Before Pitressin	After Pitressin
H. C.	10.5	1.2	.084	.078	A. M.	2.9	1.7	.67	44
C. I.	18.2	1.0	.109	.069	H. C.	2.4	2.2	.35	39
M. J.	5.7	0.3	.040	.029	E. T.	4.7	2.6	.57	47
D. B.	6.6	0.3	.007	.003	M. M.	5.5	2.8	.51	52
A. W.	11.0	1.3	.132	.127	H. L.	6.9	4.1	.55	72
A. D.	9.5	2.4	.143	.134	E. A.	3.1	2.0	.27	27
A. M.	9.6	0.9	.211	.157	G. H.	5.1	2.3	.85	51

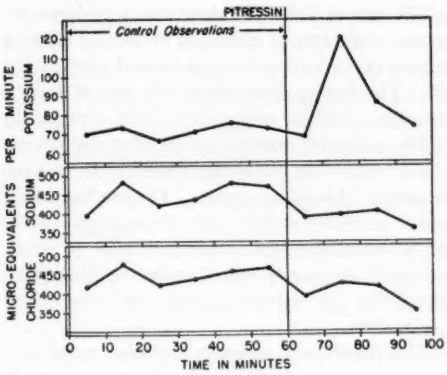

FIG. 3. THE EFFECT OF PITRESSIN (1 MILLIUNIT PER KILO INTRAVENOUSLY) ON THE EXCRETION OF SODIUM, CHLORIDE, AND POTASSIUM

There was a significant change in potassium excretion ($P < .02$).

of intravenous Pitressin on the excretion of sodium, chloride, and potassium. The sodium and chloride excretion in this group previous to Pitressin was approximately three times as large as in the water diuresis group. The excretion of sodium and chloride was not significantly changed by Pitressin. Potassium excretion showed a highly significant increase in the group from a mean of 70 ± 10 microequivalents per minute to 120 ± 14 in the first 10–20 minutes following Pitressin administration. In the two following 10 minute periods, potassium excretion began to return toward the control values. Observations on the effect of Pitressin on potassium excretion after water loading were not made.

Effect of sodium chloride excretion on water excretion after Pitressin. Table III compares the urine volume after Pitressin in two groups of individuals. Group A had received a water load of 20 cc. per kilo of body weight, and Group B had been given 0.8–0.9% NaCl solution as described above. The chief distinguishing features between the two groups so far as this point is concerned is that the water-loaded individuals were excreting relatively small amounts of salt in a large volume of water and the group who had received intravenous saline were excreting considerably larger quantities of salt in smaller amounts of water. Observation periods were 30 minutes before Pitressin and 30 minutes after Pitressin. From the previous studies, this was felt to be within the limits of the period of maximal antidiuresis. In all cases, a 10 minute period was allowed to elapse after the administration of Pitressin to correct for renal tubule to bladder delay. The dose of Pitressin in the saline group was constant, 1.0 milliunits per kilo. In the water group in some cases, it was less, but never more. The mean urine minute volume expressed in cc./min. for the 30 minute period in the saline group following Pitressin was 2.5 ± 0.29, and for the water group, 1.06 ± 0.27. Comparing these two groups statistically, the difference is of significance with a p value equal to 0.01. In terms of Pitressin effect, the experiment was weighted against the results because in some of the water experiments, Pitressin dosage was less than 1.0 milliunit per kilo. We take these results to indicate that changes in sodium excretion of a degree that is well within the average physiological range, has a modifying influence on the antidiuretic effectiveness of Pitressin.

Effect of Pitressin on renal hemodynamics. In dogs and rats no consistent effect has been found on renal blood flow and glomerular filtration rate (8, 9). Table IV shows the effect of intravenous Pitressin on these same functions in normal subjects. All studies were prepared and carried through under identical conditions. All subjects were given a similar amount of water to drink previous to starting the clearances (20 cc. per kilo of body weight) to suppress endogenous ADH. Following the initial control period (two clearances), all received intravenous Pitressin in the dose of 1 milliunit per kilo of body weight. A pe-

TABLE IV

The effect of intravenous Pitressin on renal plasma flow and glomerular filtration rate in normal subjects

Subj.	C_{IN}. (cc./min.)		C_{PAH} (cc./min.)	
	Before	After	Before	After
R. T.	131	116	752	718
M. C.	141	132	650	587
A. C.	113	117	606	634
L. C.	102	106	472	455
H. R.	111	109	538	459
R. T.	115	118	738	674
L. H.	115	101	518	470
E. M.	111	110	539	381
M. B.	106	112	416	429
D. W.	85	99	504	556
Mean	113	112	573	536
S. D.	15.2	9.5	111	114
S. E.	4.8	3.0	35.2	36.1

riod of 10–15 minutes was allowed before starting the first post-Pitressin period to allow for the mechanical effects of an abrupt change in urine volume to wear off. Then two or more additional clearance periods were carried out, each preceded by a similar dose of intravenous Pitressin to insure continuity of Pitressin effect. There was no demonstrable change in renal plasma flow and glomerular filtration rate.

GENERAL DISCUSSION

The data recorded here are consistent with the view that the physiologic effects of small doses of Pitressin on the excretion of water, sodium and chloride are quantitatively and qualitatively similar to those caused by endogenously produced ADH. Observations of others comparing the antidiuresis caused by Pitressin with that produced by smoking support this thesis (10).

The effect of Pitressin on electrolyte excretion in the dog has not been consistent. O'Connor (3) found no effect on chloride excretion. Anslow and his colleagues (7) and Sartorius and Roberts (11) have shown that under certain conditions Pitressin causes a definite increase in sodium and chloride excretion in the dog. However, since in the latter studies Pitressin apparently increased glomerular filtration rate, these experiments probably cannot be directly applied to man.

In the experiments reported here there was no effect on the excretion of sodium and chloride in the water-loaded subjects receiving inulin and PAH, and in the salt- and water-loaded subjects who did not receive inulin and PAH. In the water-loaded group who received no inulin or PAH, there was a slight, but consistent, decrease in sodium excretion. The salt- and water-loaded subjects had a lower urine flow before Pitressin and a higher urine flow after Pitressin than the water-loaded group. It has been reported that Pitressin in man produces no change in the excretion of sodium chloride (10) and that it decreases the excretion of sodium slightly in the water-loaded subject (12). The latter authors believe that the reduction in the excretion of sodium is due to the low rate of urine flow which causes sodium to be more completely reabsorbed. Our data fit nicely into this hypothesis as the retention of sodium occurred only when the average excretion of water was reduced to 1 cc./min.

The data in Table III show that a moderate increase in the rate of excretion of sodium chloride influences the volume of urine formed after Pitressin. The factors determining the rate of sodium chloride excretion seem to be little changed by ADH, but under maximal ADH effect the amount of urine formed is greatly influenced by the amount of sodium chloride excreted. The mechanism by which increased sodium chloride excretion limits the antidiuretic effect of ADH remains to be determined. A simple osmotic effect of the salt rejected by the tubules seems the most likely explanation.

This dependence of water excretion on salt excretion has been studied in a different manner by Rosenbaum, Nelson and Strauss (13). They found a decreasing water excretion in the presence of a constant water load despite a constant filtration rate with the only variable being a diminishing sodium chloride excretion. Their results show that a maximum urine flow cannot be maintained without a considerable excretion of salt.

The increase in potassium excretion following Pitressin in the salt-loaded subjects was striking and immediate and suggests that it is caused by a change in renal tubular function. Assuming that Pitressin does affect the tubular mechanism responsible for potassium excretion, two possibilities exist since as Berliner, Kennedy and Hilton have shown potassium is both reabsorbed and excreted by the tubules (14). The tubular transport mechanism for reabsorption may be inhibited or the excretory mechanism intensified.

CONCLUSIONS

1. In normal subjects rapid intravenous infusion of saline with a concentration of 125–146 millimols NaCl produced a diuresis within 30 minutes which in terms of urine volume and sodium and chloride concentration is indistinguishable from a water diuresis.

2. After a short period of time this is succeeded by an abrupt antidiuresis due to posterior pituitary activity. Sodium and chloride excretion during this period is unchanged.

3. A comparison of the effects of Pitressin given intravenously and endogenously produced ADH was made in subjects receiving 0.8 to 0.9% saline solution. The effects on the excretion of water, sodium and chloride are similar.

4. Commercial ADH (Pitressin) given intravenously in small doses causes a slight decrease in the excretion of sodium in water-loaded subjects in whom urine flow is reduced to around 1 cc./min. No effect was demonstrable in water-loaded subjects receiving inulin and PAH for clearance studies or in salt-loaded subjects. After salt loading Pitressin caused a transient increase in the excretion of potassium.

5. The urine volume after intravenous Pitressin is influenced by the excretion of amounts of sodium and chloride in the urine which are within the physiological range.

6. Small doses of Pitressin given intravenously have no effect on glomerular filtration rate and renal plasma flow.

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ARTIFICIAL KIDNEY FUNCTION: KINETICS OF HEMODIALYSIS[1]

By A. V. WOLF, DONALD G. REMP, JOHN E. KILEY, AND GORDON D. CURRIE

(From the Departments of Physiology and Pharmacology, Biochemistry, and Medicine, Albany Medical College, Albany, New York)

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Widening application of hemodialytic techniques in experimental medicine and therapy points up a need for data and theory which reflect the fundamental operations of these devices. The following study contributes to this subject.

METHODS

The artificial kidney of the Albany Hospital used in our experiments is a 1950 model built by Olson[2] according to the design of Merrill and his associates (1, 2). In all *in vitro* studies 26 turns of 23/32" cellophane tubing were used, providing a dialytic surface area of 21,000 sq. cm. When various prepared solutions were used in place of blood for the purpose of analysing rates of exchange of solutes and water between "blood" and bath fluid, a reservoir of 10 liters of "blood" was placed in series with the artificial kidney. The volume of bath fluid at the onset of dialysis was 100 liters in all studies and was maintained at 101° F. Essentially constant flow was maintained by adjusting the pump to a capacity for returning fluid to the reservoir which exceeded the actual "arterial" flow into the cellophane tubing while inflow was kept at a constant pressure head and regulated by an adjustable clamp.

Chemical analysis was as follows: urea (3), creatinine (4), non-protein nitrogen (5), uric acid (6), glucose (7), sucrose (8), amino acids (9, 10), chloride (11), bicarbonate (12), water (13), phenol red by photoelectric colorimetry, and sodium and potassium by flame photometry. Inorganic phosphate, inorganic sulfate, calcium, and magnesium methods are noted in a previous article (14).

SYMBOLS

A: concentration of a substance in arterial blood or other fluid entering natural or artificial kidney
a: minute rate at which arterial blood or other fluid enters natural or artificial kidney
α: a constant equal to $(B + b)/B$ for blood-to-bath transfer
B: volume of bath fluid
b: total volume of distribution of a substance in patient (or *in vitro*) and in artificial kidney, but excluding bath fluid volume
β: a constant equal to αk; the slope of the line relating ln (A–U) to time
C: clearance, equal to uU/A
γ: velocity constant of excretion for natural kidneys; equal to C/b for no-threshold substances
D: dialysance, equal to uU/(A–U)
d: relative dialysance, equal to D_x/D_{urea}
Δ: "change of"
e: base of natural logarithms, equal to 2.718
K: a constant representing the total solute content of the hemodialytic system, bA + BU; equal to bA_o for substances initially absent from bath fluid
k: a constant equal to D/b
n: number of changes of bath fluid during a hemodialytic procedure
R: concentration of a substance in renal venous blood or other fluid leaving natural or artificial kidney
S: average concentration of solute throughout the length of cellophane casing during flow
t: time in minutes unless otherwise specified
U: concentration of a substance in urine from natural kidneys, or in bath fluid of artificial kidney
u: minute rate of urine flow from natural kidneys; a value denoted by B/t in computing rate of solute exchange
V: volume of blood or other fluid contained in a given segment of artificial kidney excluding bath
x: "a given substance"

INTRODUCTION

During hemodialysis all substances freely filterable through the cellophane membrane have thresholds determined conjunctively by the volume and composition of the bath fluid and by the volume and composition of the blood and body fluids. If bath fluid is replaced at frequent intervals, any substance present in the plasma but not in virgin bath fluid acts as if it were a no-threshold substance, the more so as the frequency of replacement is greater. Equations which describe the kinetics of excretion in the human kidney have parallels in equations which describe the kinetics of the artificial kidney.

Under suitable conditions many no-threshold substances in man have rates of excretion which are proportional to their plasma concentrations, and the ratio of these variables is the clearance. In the artificial kidney, rates of excretion often are proportional to the concentration gradient between

[1] This work was supported in part by a grant from the U. S. Public Health Service.

[2] Edward A. Olson, Main Street, Ashland, Mass.

TABLE I

Comparative kinetics of human and artificial kidneys

Human kidney	Artificial kidney
1. Rate of excretion is proportional to blood concentration*	1a. Rate of excretion is proportional to difference between blood and bath concentrations
2. $C = \frac{uU}{A}$ (clearance)	2a. $D = \frac{uU}{A - U}$ (dialysance)
	2b. $C_t = D \cdot \frac{A - U}{A}$
3. Clearance is relatively constant with the passage of time	3a. Clearance decreases with time unless B is infinite and U = 0. Since if U = 0, C = D, then when the solute in question is not present in bath fluid, the dialysance is numerically equal to the dialytic clearance in the virgin bath at zero time
4. $C = a \cdot \frac{A - R}{A}$	4a. $D = a \cdot \frac{A - R}{A - U}$
5. $a = \frac{uU}{A - R}$	5a. $a = \frac{uU}{A - R}$
6. Diverse substances may have diverse clearances	6a. Diverse substances may have diverse dialysances
7. $A_t = A_0 e^{-\gamma t}$*	7a. $A_t = \frac{B}{B + b} \cdot A_0 e^{-\alpha k t} + \frac{b}{B + b} \cdot A_0$†
	7b. $(A - U)_t = (A - U)_0 e^{-\beta t}$
	7c. $Ab + UB = K$; $K = A_0 b$, when $U = 0$
8. $\gamma = \frac{C}{b}$	8a. $\alpha = \frac{B + b}{B}$; $k = \frac{D}{b}$; $\beta = \alpha k$
9. $\frac{U}{A} \gtreqless 1$	9a. $\frac{U}{A} < 1$ (for blood-to-bath transfer)

* Applicable to no-threshold substances such as inulin or urea.

† For substances not present in bath initially; otherwise, $A_t = \frac{B}{B + b} \cdot (A - U)_0 e^{-\beta t} + \frac{b}{B + b} \cdot A_0 + \frac{B}{B + b} \cdot U_0$. When U = 0 this equation reduces to 7a; when B = infinity and U = 0, equation 7a reduces to 7.

the plasma and the bath fluid at any instant. This implies that, at constant blood flow in the artificial kidney, the clearance of a substance tends to fall with time and is thus not useful as a hemodialytic parameter in the same way as it is in describing natural kidney function. The rate of excretion per unit concentration gradient between plasma and bath fluid is a parameter functionally equivalent to the clearance in natural kidneys, and is called here the *dialysance*, D. It is defined specifically as the minute rate of net exchange of a substance between blood and bath fluid per unit blood-bath concentration gradient. Although it varies with blood flow and surface area of cellophane (1) its value is relatively characteristic for different molecular and ionic species.

Table I compares the kinetics of excretion in natural and artificial kidneys. In spite of similar mathematical formulations for certain aspects of both human and artificial kidneys, their principles of excretion differ. In the human kidney, excretion depends primarily upon a filtration process coupled or not with tubular influence; in the artificial kidney, excretion is primarily by diffusion coupled perhaps with some filtration. It is unnecessary to elaborate the derivations of the equations indicated in the table. Some of these have been considered elsewhere (15). The basic assumptions include: *1*) the differential $dA/dt = -k(A-U)$ which is justified by most of our experimental findings, and *2*) the statement of constant solute content in the system, $bA + BU = K$. We shall not note here equations which describe the effects of steady inputs of material into the system, as exampled by urea formation. Omission of this factor, which has often a high degree of uncertainty, actually makes little difference to the results to be described, although its consideration serves to account in some measure for the discrepancy observed (*2*) between the amount of urea recovered from the

bath fluid and the corresponding drop in plasma concentration in the course of therapy.

While some information concerning dialysance may be obtained from hemodialysis in patients, much is precluded because the bath fluid contains materials, *e.g.*, sodium, in substantially the same concentration as in plasma. In this case accurate determination of extraction fraction, excretion or retention, or dialysance is not practicable. Thus, a series of *in vitro* experiments have been performed in which prepared solutions were dialysed against water or other prepared solutions, and advantage taken of analytically large gradients. It has been found that different materials have different characteristic dialysances; and the concept of the *relative dialysance*, or the ratio of the dialysance of a given substance to that of urea measured at the same time, has been introduced (Table II; Figure 3). The relative dialysances of electrolytes are influenced by the requirements of electroneutrality and Donnan equilibria, as well as by the diffusion-accelerating and diffusion-retarding influence of other solutes, but useful facts can be discovered notwithstanding.

The dialysance of water is not considered here. Of more importance to considerations of water balance is the positive or negative clearance of water when specified quantities of osmotically effective materials are contained in bath fluid and blood, but no means at our disposal provided accurate measurement of absolute water exchange.

TABLE II

Average dialysances (D) and relative dialysances (d) obtained from in vitro experiments at a "blood" flow (a) of 500 cc./min.

A dialysance listed here may be plotted as a point on the graph of Figure 3 and a curve drawn between the origin and this point in conformity with the other curves. The drawn curve approximates to the actual dialysance-flow curve for that substance. The ratio D_x/a, either from this table or from any point on a dialysance-flow curve, is numerically equal to C_x/a at zero time after bath fluid contacts cellophane and therefore to the extraction fraction, $(A - R)/A$, which would be found for x when it is absent from a virgin bath ($U_x = 0$). D/a varies with a. For all substances freely diffusible through the cellophane, D/a as well as d approaches unity as a approaches zero.

The number of determinations includes "instantaneous" values of D_x and d_x obtained from the relations $D_x = a(A_x - R_x)/(A_x - U_x)$ and $d_x = D_x/D_{urea}$, as well as from $D_x = D_{urea}(\beta_x/\beta_{urea})$. From these, dialysance-flow curves as in Figure 3 were constructed and the values of D at a = 500 cc./min. were taken for this table.

Substance	D	d	D/a	Number of determinations
Chloride	310	1.03	0.61	13
Urea	300	*1.00*	0.60	16
Potassium	300	1.00	0.60	13
Sodium	235	0.78	0.47	15
Creatinine	169	0.56	0.34	15
Bicarbonate	168	0.56	0.34	2
Tryptophane	150	0.50	0.30	1
Alanine	148	0.49	0.30	6
Uric acid	135	0.45	0.27	12
Glucose	120	0.40	0.24	13
Calcium	60–152	0.21–0.51	0.12–0.30	8
Magnesium	100	0.33	0.20	10
Glutamic acid	96	0.32	0.19	1
Sulfate	96	0.32	0.19	9
Inorganic phosphate	92	0.31	0.18	7
Sucrose*	67	0.22	0.13	7
Phenol red	33	0.11	0.07	5

* In calculating transfers of material from bath to blood by means of equations of the type in Table I (7a, 7b), substitute U in place of A, b in place of B, and B in place of b, obtaining for example,

$$U_t = \frac{b}{B+b} \cdot U_0 e^{-\frac{B+b}{b} \cdot \frac{Dt}{B}} + \frac{B}{B+b} \cdot U_0.$$

With $D_{sucrose}$ = 67 cc./min., b = 14,000 cc., B = 100,000 cc., and U_0 = 1.000 per cent sucrose in the bath at zero time, we find at t = 60 min. that U_t = 0.965 per cent. This means a movement from bath to blood of 35 grams of sucrose in one hour.

The formula $D = a(A - R)/(A - U)$ for dialysis in the bath-to-blood direction reduces to $D = aR/U$ where A = 0. R/U for bath-to-blood transfer corresponds to the extraction fraction, $(A - R)/A$, for blood-to-bath transfer.

PROCEDURES AND RESULTS

I. Volume of blood, rate of flow, and circulation time in the artificial kidney. The quantity of fluid contained in the cellophane casing depends in steady state on its rate of flow and, when the blood balance is positive or negative, upon the relation between rates of intake and output. We set our pump to operate at its maximum capacity for drawing blood from the venous side of the kidney and adjust the arterial inflow by means of a clamp, assuring a given rate of passage through the machine at any given arterial pressure. Sucking the cellophane loops to their minimal possible volume at all flows stabilizes the blood content of the patient during treatment and permits valid estimates to be made of the blood volume contained in the casing and of the average time required for the blood to pass from the proximal end of the arterial coupling to the distal end of the venous coupling. To determine extraction fractions, blood samples are drawn from these sites with proper allowance for this *circulation time*. Figure 1 relates circulation times and blood volumes in the artificial kidney to blood flow.

II. Recirculation experiments in vitro. With a reservoir containing 10 liters of solution ("blood") in series with the artificial kidney, the "blood" concentrations, extraction fractions, dialysances, etc. were determined at a given "blood" flow. The results of several studies of this type are found in Tables II and III and Figures 2–4.

III. Dialysance-flow experiments in vitro. From a reservoir containing either water alone or a prepared solution, fluid was allowed to flow at a fixed rate through the artificial kidney. Immersion of cellophane loops in a bath containing either a prepared solution or water alone was established at a determined time. Bath fluid was sampled before contact. "Arterial" fluid was sampled immediately after maximal immersion, and "venous" fluid after the appropriate "circulation time" (Figure 1). Thus, extraction fractions, either (A–R)/A or R/U, and dialysances could be calculated for "zero" time at a given flow. The results of these experiments are summarized in Tables II and III and Figure 3.

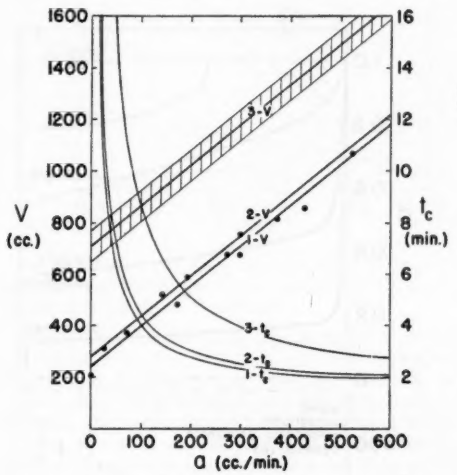

FIG. 1. RELATION OF FLUID VOLUMES, V, CONTAINED IN VARIOUS SEGMENTS OF THE ARTIFICIAL KIDNEY AND OF CORRESPONDING CIRCULATION TIMES, t_e, TO RATE OF FLOW THROUGH THE MACHINE, a

Black dots represent experimentally determined fluid volumes contained in 26 cellophane threads of the Archimedes' screw at different measured flows, after balance had been obtained in a recirculation system *in vitro*. From curve 1-V, a straight line fitting the points shown, was calculated the curve 1-t_e, since $V/a = t_e$. From measured "dead spaces" beyond the cellophane casing it was possible to estimate the intercoupling volume and intercoupling circulation time (curves 2-V and 2-t_e, respectively), and the total extracorporeal volume and extracorporeal circulation time (curves 3-V and 3-t_e, respectively). The vertically hatched region about 3-V indicates the variation in extracorporeal volume which may occur during measurement of blood flow by means of the buret ordinarily used for that purpose. Curve 2-t_e is useful in choosing the time which must elapse after sampling A, before sampling R, in the determination of extraction fractions, etc.

The equation of curve 1-V is $V = 1.6a + 240$ which indicates that for every change of flow of 100 cc./min., there is a corresponding change in casing volume of 160 cc.

TABLE III

Comparison between D, d, and D/a values from in vitro *experiments and from a patient*

Substance	a	*In vitro*			In patient (M. B.)		
		D	d	D/a	D	d	D/a
Urea	296	212	1.00	0.72	204	1.00	0.69
Non-protein nitrogen	296				177	0.87	0.60
Creatinine	296	143	0.67	0.48	123	0.60	0.42
Glucose	296	108	0.51	0.37	82	0.40	0.28
Inorganic phosphate	296	82	0.39	0.28	77	0.38	0.26

In the patient (see lower part of Figure 2 for additional information) blood flow was maintained close to and averaged 400 cc./min.; plasma flow, 296 cc./min.; b = 52,000 cc. for urea; a[illegible] = 100,000 cc. *In vitro*, b = 10,000 cc. and B = 100,000 cc. Since 25 coils of cellophane were used during the patient's run, and 26 in the other experiments, the actual D values of the patient should be increased by a small factor to be comparable (1). It is uncertain, particularly for urea, as to what value of "a" corresponds to a given total flow where whole blood is being dialyzed, since that substance so freely crosses red cell membranes.

IV. Water exchange. Without means for accurate weighing of the patient during hemodialysis it is difficult to estimate absolute water exchanges across the cellophane membrane. We attempted, however, to approximate the critical concentration gradient of glucose between plasma and bath at which no net water exchange occurred. During two hemodialyses on the same patient the glucose content of the bath was varied. A, R, and U samples were drawn at appropriate times and plasma and bath fluid were analysed gravimetrically for water content and chemically for glucose. No pyknic or osmotic corrections for transferred solute were made. Figure 5 shows how different glucose gradients cause the water content of the plasma to vary in its passage through the artificial kidney. An apparent water balance is indicated when

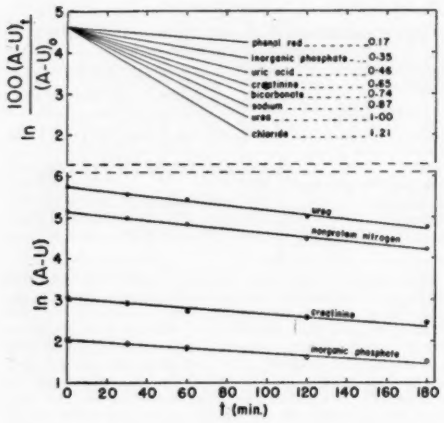

FIG. 2

Upper: In vitro results of a single experiment at a flow of a = 300 cc./min. The A-U gradient at time t was first expressed as a per cent of the gradient at zero time, giving all substances the same value of $\ln \frac{100(A-U)_t}{(A-U)_0}$ at zero time. This permits readier visualization of the varied slopes of their curves. $D_{urea} = 220$ cc./min.; $\beta_{urea} = 0.0242$ min.$^{-1}$; b = 10,000 cc.; B = 100,000 cc. Numerical values in graph are relative dialysances.

Lower: Actual values of ln (A-U) plotted against time, t, for a single hemodialysis in a 70 kg. man (M. B.). Slopes are $\beta_{urea} = 0.00595$, "β_{NPN}" = 0.00472, $\beta_{creatinine} = 0.00378$, and $\beta_{phosphate} = 0.00367$ min.$^{-1}$ The volumes of distribution of these materials in man (unlike the above *in vitro* experiment) are not identical so that the dialysance is in fixed relation to the β value only for a given material. Since

$$\beta = \alpha k = \frac{B + b}{B} \cdot \frac{D}{b},$$

we may solve for b = Db/(Bβ − D). Taking the average "instantaneous" D_{urea} (from Table III, containing additional data on this patient) as 204 cc./min., and β_{urea} as 0.00595 min.$^{-1}$, we calculate b = 52,000 cc., an expected magnitude. Analytical concentrations were in mg. per cent except for phosphate which was in mEq./l.

$(A - R)_{H_2O} = 0$. No claim is made that this point represents true water balance in the artificial kidney because no satisfactory analysis has been made of fluid shifts in the tri-phasic osmotic system consisting of fluid of blood cells, plasma, and bath fluid. In view of the difficulty in sampling whole blood and analysing it accurately for water, in view of the variable colloid osmotic pressure of plasma in different patients and at different times during a single hemodialysis, and in view of the variable effective osmotic pressure of plasma crystalloids and of those in bath fluid, these data have only relative value in suggesting glucose contents of bath fluid conducive to hydration or dehydration of the patient.

In Figure 5 apparent exchanges of water across the membrane are shown as a function of the average bath-plasma gradient of glucose concentration, $(U-S)_{glucose}$. This value is found from

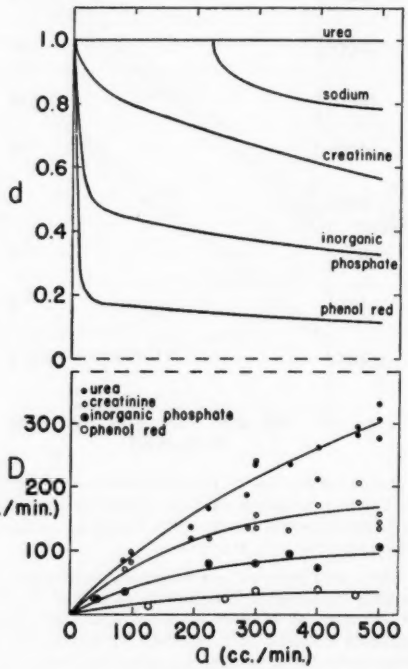

FIG. 3

Upper: Relative dialysance, d, as a function of "blood" flow, a. These curves are based upon the values of D_x/D_{urea} obtained from the lower curves. Limiting values for d are not obtainable at flows as small as here employed. The sodium curve included here but not among the lower curves points up the fact that intrinsically different dialysances may not be disclosed until relatively high flows are reached.

Lower: Dialysance, D, as a function of "blood" flow, a. These curves are based upon determinations as described in the caption of Table II. Limiting (maximal) values for D are not generally obtainable at flows as small as here employed, but for substances of low dialysance, *e.g.*, phenol red, they are approximated.

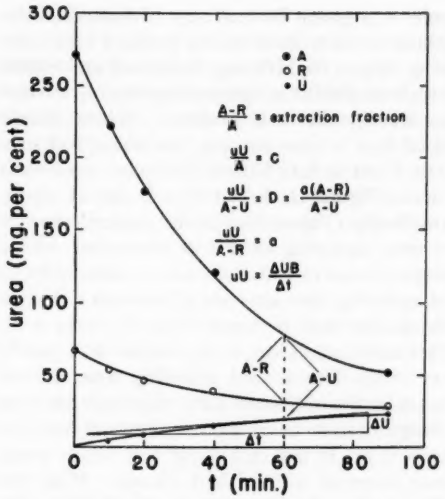

FIG. 4. RELATION OF UREA CONCENTRATION IN "BLOOD" ENTERING ARTIFICIAL KIDNEY, A, LEAVING THE KIDNEY, R, AND IN BATH FLUID, U, TO TIME, t

Results are of a recirculation experiment *in vitro* where a = 300 cc./min., b = 10,000 cc., and B = 100,000 cc. Illustrated is the basis for calculation at t = 60 min. of "instantaneous" urea excretion rate, extraction fraction, clearance, dialysance, and flow. $\Delta U/\Delta t$ is the slope of the U curve at t = 60, and $\Delta UB/\Delta t$ is the minute rate of excretion (units conforming to those of A and R) for which the term uU may be substituted. All curves approach a common asymptote. At zero time the clearance, whose value falls with time, is equal to the dialysance whose value is independent of time.

the formula

$$(U - S)_{glucose} = \frac{(R - A)_{glucose}}{\ln \frac{(U - A)_{glucose}}{(U - R)_{glucose}}}$$

based upon the exponential decrease of the glucose gradient along the cellophane path. Evidence for this type of decrease is afforded in information given by Merrill and his colleagues (1) on the relation between the extraction fraction and the number of coils of cellophane. U–S may also be found by using an "A bar" table (15). S is approximated for poorly extracted materials by $(A + R)/2$ or $\sqrt{AR}$.

A precise theoretical and practical solution to this problem of water balance seems remote so long as we deal with materials like glucose in the bath. Sucrose, having a smaller dialysance, offers possibilities which have not been examined therapeutically but it is far from ideal.[3] The cryoscopic method of Merrill and his associates (1) for adjusting fluid balance has no theoretical validity since the osmotic pressures in blood and bath do not reflect the fugacity of the fluids in these phases (15).

The relationship between the water clearance and blood flow at given U–S gradients of osmotically effective materials has not been carefully studied. Presumably where large U–S gradients exist, the water clearance is proportional to flow when flow is extremely small, *i.e.*, when the maximum possible clearance of water from plasma occurs. Where flow (ignoring colligated pressure effects) considerably exceeds the water clearance the two

[3] We find glucose solutions to be only ca. 0.75 times as effective as isosmolar sucrose solutions in causing osmosis at intermediate flows. Presumably this relates to the greater relative dialysance of glucose.

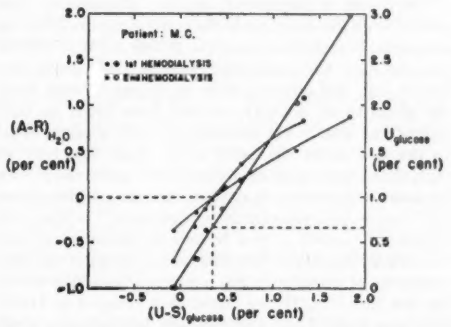

FIG. 5. EFFECT OF GLUCOSE IN BATH FLUID ON WATER CONTENT OF PLASMA DURING HEMODIALYTIC TRANSIT

$(A\text{-}R)_{H_2O}$ is the difference between the water concentration (g. water per 100 g. plasma) of plasma entering and plasma leaving the artificial kidney. $(U\text{-}S)_{glucose}$ is the average bath-plasma concentration gradient of glucose determined as indicated in section IV of the text. $U_{glucose}$ is the bath concentration of glucose. The curved lines fitting small circles relate $(A\text{-}R)_{H_2O}$ to $(U\text{-}S)_{glucose}$. The straight line fitting large circles relates $(U\text{-}S)_{glucose}$ to $U_{glucose}$. The point of apparent water balance, where $(A\text{-}R)_{H_2O} = 0$, is thus found on the interrupted line at $(U\text{-}S)_{glucose} = 0.36$ per cent and at $U_{glucose} = 0.66$ per cent. Plasma protein at t = 0 ranged from 4.9 to 5.3 per cent. Blood flow: first hemodialysis, 130–158 cc./min.; second hemodialysis, 188–240 cc./min. Water concentration in acalcic, aglycic bath fluid: first hemodialysis, 99.1147 per cent; second hemodialysis, 99.1455 per cent. Hematocrit: ca. 0.26.

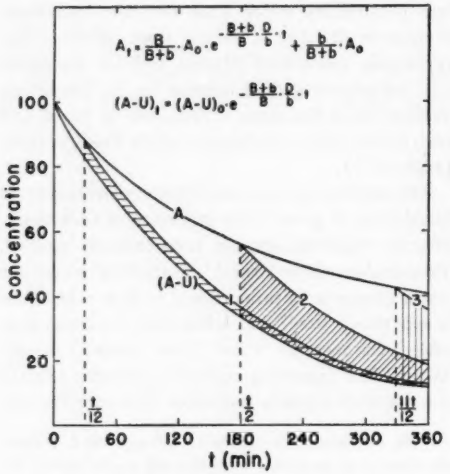

FIG. 6. GRAPHIC DETERMINATION OF OPTIMAL TIME FOR CHANGING BATH FLUID

The curves A (uppermost) and A-U (lowermost) were plotted from the equations in the graph using the following constants: D = 200 cc./min.; b = 50,000 cc.; B = 100,000 cc.; and A_0 = 100 concentration units. In a given time period, *e.g.*, 360 minutes, with no change of bath fluid, the quantity of material removed from blood to bath (containing none of the material at t = 0) is proportional to the area under the curve A-U. If at time t/12 we replace the bath with fresh fluid, A-U is increased from its value with respect to the first bath to a higher value, $(A-U)_{t/12} = A_{t/12}$, since for the second bath, U = 0 at t/12. A new A-U curve, 1, now bounds an increment of area denoted by horizontal hatching which measures the augmentation of excretion in 360 minutes. Instead of changing the bath at t/12, we might have changed at 11t/12 obtaining curve 3 and the vertically hatched area would have measured the augmentation of excretion. The obliquely hatched area under curve 2 derived from a bath change at t/2 is the maximal possible, hence t/2 is the optimal time for changing the bath once during a hemodialytic run whose total operating time is t.

variables become dissociated, the latter becoming essentially dependent only upon the U–S gradient. Evidence for this is suggested in the dissociation of the dialysances (and, therefore, the clearances) of other poorly extracted materials (*e.g.*, phenol red) from flow (Figure 3).

V. Dialysances in man. The effect of hemodialysis on urea, non-protein nitrogen, creatinine, and inorganic phosphate in a three hour period is shown in Table III and Figure 2.

VI. Optimal time for change of bath fluid. In order to augment the exchange of hemodialyzable solutes in the artificial kidney during a total operating time, t, the bath may be drained and refilled with fresh fluid at an intermediate time, t_1, increasing thereby the A–U gradient. Where dialytic blood flow is kept constant, the removal of urea from blood to bath follows closely an exponential curve (Figures 2, 4, and 6). It can be shown graphically (Figure 6) that the maximal removal of urea (ignoring its rate of formation) with a single change of bath fluid occurs when $t_1 = t/2$. All operating time intervals, t_1', between n changes should therefore be equal,[4] and $t_1' = t/(n+1)$. This holds regardless of the values of A and U at t = 0. For a total operating time, t = six hours, it was estimated (16) that with one bath change at three hours the urea removal from the body is ca. 16 per cent above that which would have occurred with no bath change. With two changes (two and four hours) the increment is ca. 20 per cent. With an infinite number of changes the calculated increment is ca. 31 per cent. These particular approximations were for a system having a bath volume of 100 liters, a 70 kg. man, and a urea dialysance of 175 cc./min.

[4] We are indebted to Dr. Ralph A. Beaver for providing a general mathematical solution for the problem.

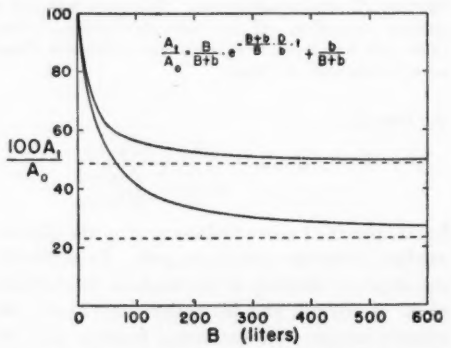

FIG. 7. THE PER CENT OF SOLUTE REMAINING IN THE BLOOD, $100A_t/A_0$, AS A FUNCTION OF THE VOLUME OF BATH FLUID, B

Arbitrary constants for the indicated equation in the graph are t = 360 min.; b = 50,000 cc.; upper curve, D = 100 cc./min.; lower curve, D = 200 cc./min. The upper and lower horizontal lines denote the value of 100 A_t/A_0 when B is of infinite volume for the upper and lower curves, respectively.

VII. The effect of bath fluid volume on total exchange. In Figure 7 is plotted the percentual decrements calculated to occur in the plasma concentration of a substance at two different dialysances (flows) after six hours of dialysis against an unchanged bath of given volume. Increasing the volume of the bath fluid beyond certain limits carries no clear advantage to total removal (which bears upon theoretical aspects of counterflow dialysis systems which may keep U close to zero, and which effectively create a bath of semi-infinite volume). It can be shown, for example, that a single change of a 100 liter volume of bath fluid at the optimal time may, in a certain total operating time, have as good an effect in lowering blood concentration of urea as if a 300 liter volume had been used without change in the same operating time.

DISCUSSION

The fact that the circulating fluid volume in the cellophane casing is proportional to the rate of flow has practical importance to the technique of hemodialysis. If one seeks to minimize fluctuations in a patient's blood volume during treatment he will bear in mind that rapid changes of flow through the cellophane cause rapid changes in blood volume. An increment in flow of 100 cc./min. causes approximately 160 cc. of blood to leave the patient for the machine; vice versa for a decrement in flow.[5] Further, proper attention to volume-flow relations enables one to reduce the likelihood of pulmonary edema which may occur if, for example, at the end of a run at 400 cc./min., the arterial inflow to the machine is stopped and the machine allowed to disgorge its contained blood rapidly into the patient. Even in the absence of excessive dilation of terminal loops a sudden plethora of 720 cc. of blood could be produced in less than two minutes. If terminal loops are dilated, much more blood than this can be forced on the patient. Watching a machine with no arterial inflow, but with dilated loops, return blood to a patient gives the illusion that it is only the swollen loops which are being drained off. Actually the engorged loops are being fed constantly until the casing proximal to them has become relatively empty. Operation of the artificial kidney without unnecessary dilation of terminal loops greatly assists the operator in stabilizing and regulating volumes and flows.

Although solutions containing several ions with concentrations up to 150 mEq./l. for sodium and chloride, and lesser values for other ions, have permitted reasonably reproducible determinations of dialysances, it is realized that different conditions engender other results. For example, Table II suggests that potassium is a highly diffusible and highly permeable ion ($d_K = 1.00$) and phosphate a poorly diffusible and/or permeable one ($d_{phosphate} = 0.31$). But where KH_2PO_4 was the only electrolyte in the "blood" in one test, it was found initially that $d_K = 0.65$ and $d_{phosphate} = 0.40$. This points up the interdependence of ions in a system where their freedom of movement is limited by coulomb forces and by the paucity of other ions whose presence could serve to increase the freedom of the inherently more mobile ones. The dialysance of one ion, in a mixture of several, has occasionally been observed to change appreciably as dialysis proceeded. And pH changes, which could be expected to occur under these conditions, have been observed. Nevertheless the study of dialysance is fruitful. It enables us to appraise potential transfers of materials in physiological and clinical tests; it points up the dialytic process as one of true "separation" not merely between colloids and crystalloids, but among diverse ionic and molecular species; and it enables us to make estimates of rates of tranfer as a function of molecular weight[6, 7] and electric charge.

SUMMARY

1. An artificial kidney (Brigham-Kolff type) has been used to study kinetics of hemodialysis *in vitro* and in man. Its operation has been described quantitatively and parallels to human renal excretion have been formulated.

2. The concept of dialysance, or the minute rate of net exchange of a substance between blood and

[5] The practice of hemodialysis lays the basis for a study of the effects of "varivolemia" on the circulatory function.

[6] Relative dialysances of non-electrolytes obtained from the range of flows used here are not definitive in the sense of being limiting values. However, they generally decrease with increase of molecular weight.

[7] That transfers of substances of higher molecular weight are relatively independent of blood flow (Figure 3) suggests a possibility of inferring the nature of the "causative" agents of uremia by the way in which hemodialytic relief of uremic symptoms may vary with rate of blood flow.

bath per unit blood-bath concentration gradient, is introduced as a parameter in artificial kidney function corresponding to the clearance in natural kidney function. Its value is relatively characteristic for different molecular and ionic species although for any one species it increases with blood flow and cellophane surface area. Using the urea dialysance as a reference, relative dialysances have been determined under various conditions for several non-electrolytes and ions.

3. The volume of circulating fluid contained in the cellophane casing is proportional to the rate of flow. From this relation have been deduced circulation times through the machine. A method is presented for stabilizing a patient's blood volume during therapy and reducing the likelihood of occurrence of pulmonary edema.

4. The effect of various concentrations of glucose in the bath fluid on fluid balance in the artificial kidney has been studied quantitatively.

5. In order to augment maximally the exchange of hemodialyzable solutes during some total operating time, the bath may be drained and refilled with fresh fluid at an intermediate time. It is concluded that, for any given total operating time and any chosen number of changes of bath fluid, operating time intervals between changes are optimum if equal.

6. The effects of dialysance, blood flow, and bath volume on the exchange of material during hemodialysis are quantified.

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THE STUDY OF SERUM PROTEINS AND LIPIDS WITH THE AID OF THE QUANTITY ULTRACENTRIFUGE. I. PROCEDURE AND PRINCIPAL FEATURES OF THE CENTRIFUGATE OF UNTREATED NORMAL SERUM AS DETERMINED BY QUANTITATIVE ANALYSIS OF SAMPLES FROM TEN LEVELS[1, 2]

By ROY H. TURNER, JOHN ROBERT SNAVELY, WILLIAM H. GOLDWATER, MALCOLM L. RANDOLPH, CHARLES C. SPRAGUE, AND WALTER G. UNGLAUB

(From the Department of Medicine, School of Medicine and the Biophysics Laboratory, Tulane University of Louisiana, New Orleans, La.)

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The studies described in this paper and some which follow have as an objective a better description of serum lipids. The present paper represents the first detailed report of work begun early in 1948, some features of which have been published in abstract (1–5). Initially our purpose was to extend the observations of Recant, Chargaff and Hanger (6) on the influence of serum proteins upon the cephalin-cholesterol flocculation and the thymol turbidity tests. We included quantitative analyses of the centrifugate for lipids as well as for proteins. It soon became apparent that we were using a procedure which was useful in the study of the structure and function of lipid complexes.

As shown in the review by Edsall (7) there has long been evidence that a large proportion of the lipids of the serum occur not as small molecules, but as lipid aggregates, or lipoproteins, of varying complexity. The use of the ultracentrifuge, both quantitative and analytical, has a well established place in the study of serum lipids. Important contributions have been made by McFarlane (8, 9), Pedersen (10, 11), the Harvard group (12), Gofman and the Berkeley group (13–17), and more recently by others (18–20).

The Berkeley workers have effectively used both the quantity and analytical ultracentrifuge in the study of serum lipids and have established exacting technics for the use of the analytical ultracentrifuge in the description of lipid complexes by measurement of rate of flotation when suspended in a standard medium, a technic which we have not used. Principal emphasis in published studies by other workers has been on centrifugation of treated serum. In the present paper we deal exclusively with centrifugation of untreated whole serum. At present it does not seem profitable to attempt a detailed comparison between the results obtained by our procedure on the one hand and those obtained by the system of analysis introduced by Cohn and his associates (21), and the results of the flotation procedures of others. When our studies of diseased states and normal individuals under dietary stress have been presented, such comparisons will be in order. Some of the similarities of our contributions to those of others will be mentioned below in the discussion.

The perfect procedure for the study of serum proteins and lipids in the initial step would give complete separation of the different lipid and protein complexes, each entity in pure state with structure unchanged by the procedure effecting separation. We doubt if the procedure described in this communication separates any single lipid or protein complex in pure form; nevertheless, the concentration of complexes at certain levels in the centrifugate is of such a degree as to give new information concerning the structure of the complexes at that level and their concentration in the serum under study. The lipid distribution curves are reproducible within satisfactory limits with normal sera, and are significantly different in certain diseased states, especially in acute virus hepatitis, as we have already reported briefly (3) and will report later in detail. We believe our procedure has three main advantages: The manipulation of serum which effects redistribution of pro-

[1] This work was done in part under a contract from the Office of the Surgeon General, U. S. Army, and under the sponsorship of the Commission on Liver Disease, Armed Forces Epidemiological Board.

[2] Some of the data in this paper were presented at the Tenth Liver Injury Conference of the Josiah Macy, Jr. Foundation on May 21, 1951.

teins and lipids is gentle and involves minimal risk of damage to complex molecules; description of samples is by multiple quantitative methods; and an integrated picture of the protein and lipid compositions of whole serum is provided. The obvious disadvantages are that the method requires a large sample of serum, expensive apparatus and much time of highly trained workers.

MATERIALS AND METHODS

The data reported in the present paper were obtained by study of sera from 14 normal young men, either medical students or members of the laboratory staff, who were apparently in excellent health. Chemical analyses of whole serum for the constituents under study fell within the normal range as determined in our laboratory. From an original group of 16 subjects, two were eliminated because the estimations on the whole serum for neutral fat exceeded by more than 3 standard deviations the mean of the 16. Blood was drawn in the morning after a 12-hour fast, the subject usually having been instructed to eat no fat-rich food the preceding evening. The serum was stored at 4° C. until the following day, when centrifugation was performed. Commonly, three or more tubes were filled with a single serum so that several sera, including at least one normal and one abnormal, were centrifuged at one time. The Beams type air-driven ultracentrifuge was used with a rotor holding 12 plastic tubes, each of one-half inch diameter and 8.2 ml. capacity, set at an angle of 10° from the axis of rotation (22, 23). The centrifugation was performed at a speed of 800 revolutions per second, producing a mean centrifugal force of 130,000 G., for four hours, with about 23 minutes for acceleration and from 75 to 110 minutes for deceleration. The standard speed was maintained with an error of less than 1% by hand regulation guided by an oscillographic speed indicator. A vacuum of less than 0.0005 mm. Hg was maintained about the rotor, which had a temperature of about 28° C. After the rotor had coasted to a stop and had been removed from the vacuum chamber, the plastic tubes containing the centrifugate were carefully removed and sampled.

The objective in sampling was to obtain reproducible segments from the column so that centrifugate from corresponding levels of tubes of the same serum might be pooled for analysis and samples from comparable levels of different sera might be compared. Hughes, Pickels and Horsfall used the quantity ultracentrifuge for the study of the sedimentation properties of serum proteins and separated the centrifugate into six samples (24). Previously we employed a sampling technic of quick freezing of the centrifugate and cutting the frozen column on a lathe (25). However, for the work now being reported, we have used a device for sampling liquids which obviates the difficulties of thermal convection, and possible denaturation of lipoproteins by freezing. This device drives a thin pointed knife blade horizontally through the walls of a vertically clamped plastic tube and through the column of liquid centrifugate at successively measured levels in such a fashion that the blade forms a sealed bottom to

TABLE I

Principal features of centrifugate according to density zones

Descriptive term for density zones	Composition according to sampling technic and vol. in 6 tubes	Vol., % whole column	Range, % column ht.	Sp. gr. range	Physical appearance	Protein content, % total in centrifugate	Total lipids content, % total in centrifugate	Distinguishing chemical characteristics
Minimum density	No. 1 = 5.71 Top sample	12	88–100	1.000–1.004	Top is creamy	0.3	21	Rich in neutral fat
Minimum concentration	No. 2 = 7.00	14	74–88	1.004–1.010	Water clear	0.9	5	Inconstant with usual procedure; poor in proteins and lipids
Low-medium density	No. 3 = 6.05 4 = 6.12 5 = 6.05 18.22	37	37–74	1.010–1.028	Turbid band	18.0	44	High concentration all lipid constituents
High-medium density	No. 6 = 6.12 7 = 3.17 8 = 3.17 12.46	25	12–37	1.028–1.055	Clear dark yellow	40.7	22	All lipid constituents well represented except free cholesterol
High density	No. 9 = 3.17 10 = 2.70 5.87 Bottom samples	12	0–12	1.055–1.110	Clear brownish yellow	40.0	7	Phospholipids and neutral fat only important lipids; high concentration globulins

the segment of tube above that level (26). The contents of the tube above the blade are drawn into a syringe through a No. 20 gauge needle. The theoretical volumes of our 10 standard samples of centrifugate are shown in Table I.

During centrifugation the components of serum tend to form cylindrical layers about the axis of rotation. During deceleration and while the tubes stand vertically before sampling, these layers assume a new transverse arrangement in the tubes. The success of sampling depends upon this reorientation. That a rearrangement does occur is evident from gross inspection. The top cream layer and various bands of color and turbidity at different levels, sometimes seen as low in the tube as 15% column height, are sharply defined and horizontal. Such a reorientation of layers is not always perfect as is shown by the observation during sampling of highly viscous protein material concentrated at the peripheral side of the bottom of the tube. In a few instances the bottom sample has been divided by cutting vertically and the outer fraction showed significantly higher concentration of protein than the inner. On the side of the tube toward the axis of rotation there is sometimes observed a thin projection downward of part of the creamy material from the top of the column. This has not been observed after prolonged centrifugation.

CHEMICAL METHODS

Total protein and albumin values were based on micro-Kjeldahl nitrogen determinations (27), using a factor of 6.25 to convert nitrogen to protein. Albumin-globulin separations were accomplished by saturation with $MgSO_4$, using the technic employed by Popjak and McCarthy (28), and albumin nitrogen was determined on the filtrates. Non-protein nitrogen determinations on tungstic acid filtrates of a series of samples of ultracentrifugate showed all to have concentrations of 30 to 40 mg.%, with no appreciable deviations from normal values for whole serum. Accordingly, a corresponding value of 0.2 gm.% was uniformly subtracted from the total protein and albumin values obtained by total nitrogen determinations.[2]

Specific gravity determinations were done by the falling-drop method of Barbour and Hamilton (29). Whenever concentrated protein solutions from the lower layers of the centrifugate were diluted with 0.85% NaCl solution to facilitate their manipulation, the specific gravity of the diluted specimen was corrected for the saline dilution, using 1.0045 as the density of the saline, a value obtained by pycnometer and falling-drop technics.

All lipid analyses were performed on extracts of the samples in 24 volumes of ethanol-ether, 3:1 by volume. Cholesterol determinations were done by the Schoenheimer-Sperry technic (30), with some modifications suggested by Sobel and Mayer (31). Ester cholesterol values were converted to cholesterol esters via the factor 1.67, assuming a molecular weight of 277 for average serum fatty acids (32). Lipid phosphorus was determined on aliquots of the lipid extract evaporated to dryness in a hot water bath and digested with perchloric acid (33), with color development according to the method of Fiske and SubbaRow (34). A factor of 25 was used to convert values for lipid phosphorus to phospholipid. Fatty acid determinations were done following a method (35) based on the Man and Gildea modification (36) of the older Stoddard and Drury technic (32). To calculate neutral fat, there have been assumed to be 2 mEq. of fatty acids per mole of lipid phosphorus, and 1 mEq. per mole of cholesterol ester. The total of these values is subtracted from the figure for total fatty acids, to give neutral fat fatty acid concentrations in milliequivalents. Multiplying this quantity by 290 gives the milligrams of neutral fat, assuming the average serum fatty acid molecular weight of 277. By expressing as neutral fat the fatty acids which are found in excess of those attributable to the phospholipids and cholesterol esters determined, we do not exclude the possibility that significant quantities of fatty acids may be present in some other form, such as free fatty acids, or else not measured by the method used, as would be true for short-chain water-soluble fatty acids. Total lipid values are a summation of the individual lipid concentrations—free cholesterol, cholesterol esters, phospholipids, and neutral fat.

GRAPHS

The graphs (Figures 1 and 2) present the mean concentrations of different constituents versus volume of centrifugate, as measured from the bottom of the tube. The plotted points represent the mean values in each of the 10 standard samples per serum and are placed at the midpoint in theoretical volume for each sample. Smooth curves have been drawn to yield the same content in each sample as the measured mean value for that sample, but the curves do not necessarily pass through the plotted points. Concentrations in the top sample, which is far from homogeneous because it contains the lipid-rich cream layer, are represented by rectangular bars. The content of a given material of any sample or selected volume of the tube is directly proportional to the area under the appropriate curve and between the chosen limits of volume. Water-soluble compounds unaffected by ultracentrifugation would appear on such graphs as nearly horizontal lines, similar to one representing concentration of water. This is approximately what happens with non-protein nitrogen, sodium and potassium.

At certain levels the shape of the curves we have drawn is influenced by the volume of the samples. This is well illustrated by the two top zones contained in samples 1 and 2, the upper one of which contains the cream layer. Our graphs present the mean concentration for the entire top sample, whereas in fact less than one-third of the No. 1 sample constitutes the concentrated creamy ma-

[2] In some instances when 0.2 gm.% was subtracted from the albumin concentration based upon nitrogen estimations in the Popjak filtrate, negative values resulted. In such cases the albumin concentrations were taken as zero, and the globulin values accordingly equal the total protein concentration.

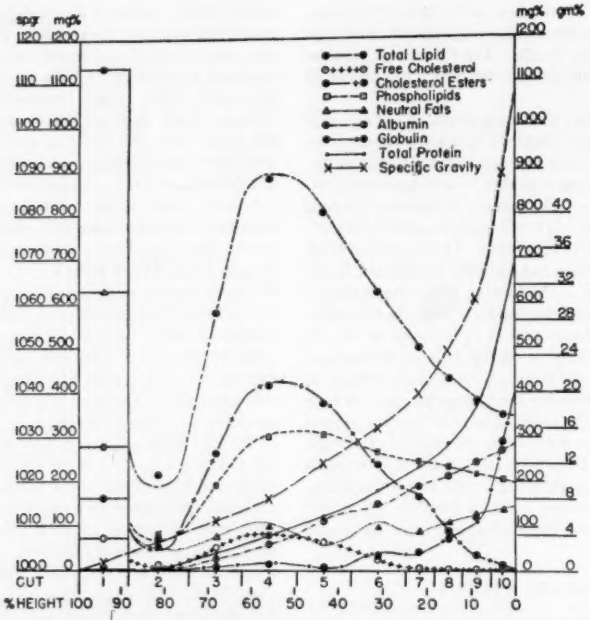

FIG. 1. PROTEIN AND LIPID COMPOSITION OF ULTRACENTRIFUGATE OF NORMAL SERUM SPUN FOR EIGHT HOURS AT 800 R.P.S. (SEE TEXT), BASED ON MEAN CURVES FOR 14 SERA

Concentrations shown in mg. per 100 ml., except for proteins which are in gms. per 100 ml. First row of figures at the bottom opposite "cut" is number of sample, the boundary of each sample being indicated by the lines between these numbers. Bottom row of figures, 0 to 100, represents either % of volume of column of centrifugate, or % of column height, assuming a flat bottom for the tube.

terial at the top of the liquid column. When we had divided sample 1 of normal serum into two equal parts, the creamy upper half was found to contain nearly all the lipid constituents and the clear lower half resembled the No. 2 sample. In some pathologic sera the clear lower part of the top sample contains a high concentration of lipids.

Because of minute leaks and coating of apparatus and glassware, our sampling technic introduces some volumetric losses, part of which may consist of material from either the upper or lower portion of a given sample. In our construction of graphs we have assumed that the volume of centrifugate lost from each sample contained mean concentrations of all the constituents measured in that sample. The magnitude of errors from this assumption is not, in our opinion, a cause for concern with our current objectives.

RESULTS AND DISCUSSION

The effects of ultracentrifugation of untreated normal serum are best visualized by inspection of the mean curves of distribution in Figures 1 and 2 and a summary of principal features of the centrifugate in Table I. The description of centrifugate in this paper, unless otherwise stated, is based on the mean curves. The greatest changes in concentration as a result of four hours of centrifugation are found at the bottom and top of the tube where globulin and neutral fat, respectively, are concentrated. As shown in Figure 2, globulin is concentrated about seven times in the bottom sample and neutral fat less than four times in the top sample. With prolonged centrifugation globulin concentration in the bottom sample may be 12 times that of the original serum and total lipids at one level are concentrated five and a half times (Figure 3).

The concentration of total proteins and, in paral-

lel, values of specific gravity increase at a progressively increased rate from the top sample with 0.3 gm.% to the bottom sample of the column with 28 gms.%, the latter concentration being about 90 times the former. The distribution curves for albumin are simpler in form than those for globulin in keeping with the evidence that albumin is the more homogeneous. In the top one-quarter of the column and in a narrower zone at the bottom globulin concentration exceeds that of albumin; as shown in Figure 2, there is a peak concentration of globulin slightly over the mid-point of the column in association with concentration of various lipids. We believe that much of these globulins in the upper two-thirds of the column are protein constituents of lipoproteins. We will present results of electrophoresis of centrifugate in a later report.

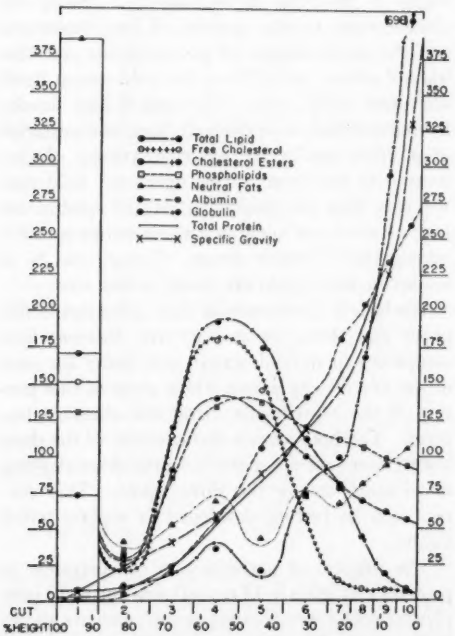

FIG. 2. SAME DATA AS IN FIG. 1, EXCEPT COMPOSITION OF THE CENTRIFUGATE IS EXPRESSED RELATIVE TO THE COMPOSITION OF THE WHOLE SERUM

In expressing specific gravity as % of original serum, only those figures to the right of the decimal point have been used.

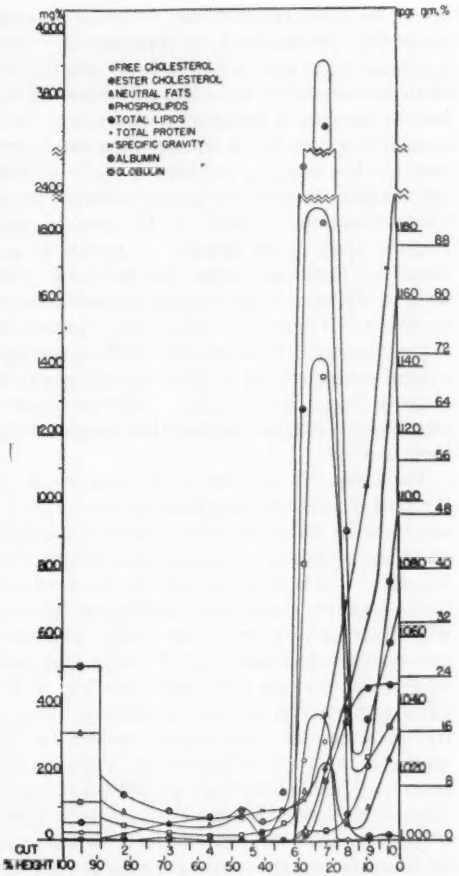

FIG. 3. PROTEIN AND LIPID COMPOSITION OF ULTRACENTRIFUGATE OF A SINGLE NORMAL SERUM SPUN FOR 16 HOURS AT 800 R.P.S.

The mean curves of distribution for the individual lipid components show varying degrees of individuality. In general the similarities in form are greater in the upper half of the column while extreme differences are found in the lower half. Shown in Table I are the distinctive physical and chemical features of five zones of increasing density from the top of the column: *1*) Minimum density. *2*) Minimum concentration. *3*) Low-medium density. *4*) High-medium density. *5*) High density. The zone of minimum density contains 21% of the total lipids and consists of two

layers, the upper and narrower of creamy material containing chylomicrons, or lipomicrons. The lower and larger layer is a clear liquid, which in its chemical composition resembles that described below for the zone of minimum concentration. Because it is not feasible to separate these two layers exactly while sampling and because we know that the sample consists of two grossly different parts, we represent the contents of this zone by rectangular areas in our graphs. It cannot be assumed for pathologic serum that the lower clear layer in this zone is low in lipid concentration as we have so far found it in the normal. Neutral fat is the principal lipid component but there are significant concentrations of both free and esterified cholesterol and phospholipids. There are moderately exact mathematical interrelationships among these four lipids.

The distinctive features of the centrifugate in the zone of minimum concentration no doubt result from the movement out of this zone of nearly all of the lipids and proteins in one direction or the other. The zone may be greatly broadened and the composition simplified by prolonging the centrifugation for more than four hours. One normal serum has been centrifuged for eight hours and another for 16 at our usual speed (see Figure 3). Eight samples from the zone of minimum concentration of these two centrifugates showed the following mean values: specific gravity 1.006 (maximum 1.009, minimum 1.002), globulins 0.15 gm.% (maximum 0.20, minimum 0.10), phospholipids 29 mg.% (maximum 48, minimum 18), neutral fat 46 mg.% (maximum 84, minimum 21) and insignificant concentrations of cholesterol and albumin. In five of the 14 normal sera subjected to our standard technic, which showed lowest concentrations of lipids in the No. 2 samples, this zone had a composition which resembled that of the broadened zone, having the same mean specific gravity, similar concentrations of free cholesterol and neutral fat, slightly higher values for phospholipids, but considerably higher for cholesterol esters. We believe that prolonged centrifugation reveals distinctive characteristics not discernible when the usual technic of centrifugation is employed.

Two main possibilities are suggested to explain these findings: One is that the lipids remaining in this zone after prolonged centrifugation consist of small molecules, little influenced by centrifugation. If this proves to be the correct explanation, distribution throughout the column would give greater quantities of this material than are now apparent. The other possibility is that large molecules containing these lipids have the observed distribution because of their density. Lindgren, Elliott, and Gofman have published in graphic form the results of similarly prolonged centrifugation of untreated serum (17). Our graph in Figure 3 shows a number of similarities to and differences from theirs.

The two zones of medium density meet at the level where the specific gravity of the centrifugate is that of normal serum. The zone of low-medium density is the zone of largest volume and contains 44% of the total lipids of normal serum and a still greater proportion of phospholipids and cholesterol, particularly the free form. The lipids found in the zone of high-medium density are characterized by the sparsity of free cholesterol and the predominance of phospholipids and cholesterol esters, and 22% of the total serum lipids are found in this zone. The zone of high density is characterized by extremely high concentration of proteins and by the extreme sparsity of cholesterol in any form. The significant lipid constituents here are phospholipids and neutral fat, both of which are found in concentrations approximating that of whole serum. Twenty-one % of the serum total lipids are found in this zone.

We believe from study of data presented in this paper that there are at least five different lipid complexes in normal serum, one entity for each of the five density zones, which gives to that portion of the centrifugate distinctive chemical features. The lipoproteins characteristic of the three lowest zones show a marked degree of overlapping distribution among the three zones. This concept will be further developed in reports which follow.

The degrees of variability in concentration of proteins and lipids in 14 normal sera and in 10 samples for each corresponding centrifugate are shown in Table II. The frequency distribution curve of concentrations at each sampling level usually shows skew because one or more high values causes a long tail on the curve, as is commonly observed in statistical analyses of a small number of samples. The standard deviation of the mean con-

TABLE II

Mean, standard deviation of the mean, maximum and minimum values of measurements made on whole serum and 10 samples of centrifugates of sera from 14 healthy young men

In column under "S" are values for whole serum and beneath the numbers 1–10 (in columns 1–10) are values for centrifugate samples serially from top to bottom of the column.

		S	1	2	3	4	5	6	7	8	9	10
Sp. gr.	Mean	1.027	1.002	1.007	1.011	1.016	1.024	1.032	1.040	1.049	1.061	1.089
	S.D.	0.001	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.005	0.004
	Max.	1.029	1.007	1.009	1.014	1.021	1.027	1.036	1.044	1.053	1.072	1.097
	Min.	1.026	0.997	1.004	1.008	1.013	1.021	1.028	1.036	1.045	1.055	1.083
Total prot.* *gms. %*	Mean	7.25	0.21	0.47	1.69	3.72	5.97	8.78	11.60	14.29	18.35	28.23
	S.D.	0.30	0.14	0.30	0.73	0.63	0.47	0.56	0.76	0.81	1.45	1.63
	Max.	7.67	0.46	0.98	3.29	4.81	6.52	9.55	12.74	15.64	21.64	30.68
	Min.	6.90	0.01	0.10	0.85	2.70	5.13	7.96	10.54	12.79	16.81	25.84
Alb.,† *gms. %*	Mean	5.16	0.13	0.25	1.25	2.94	5.46	7.39	9.41	10.64	12.16	13.50
	S.D.	0.35	0.18	0.17	0.65	0.73	0.37	0.45	0.60	1.13	1.24	1.68
	Max.	5.70	0.53	0.53	2.52	3.92	5.91	8.11	10.76	12.58	14.74	16.33
	Min.	4.61	0.00	0.02	0.47	2.00	4.61	6.75	8.94	9.12	10.84	10.90
Glob.,† *gms. %*	Mean	2.11	0.16	0.23	0.42	0.74	0.46	1.50	2.10	3.62	6.07	14.47
	S.D.	0.41	0.05	0.10	0.14	0.35	0.27	0.37	0.66	1.35	0.99	1.82
	Max.	2.74	0.45	0.45	0.77	1.27	0.82	1.95	3.30	5.28	7.50	17.80
	Min.	1.60	0.00	0.06	0.23	0.29	0.13	0.99	1.36	1.92	4.41	12.42
A/G ratio	Mean	2.55	0.29	1.39	2.93	5.41	13.83	5.46	4.85	3.52	2.05	0.95
	S.D.	0.62	0.56	0.75	1.04	3.75	12.81	1.77	1.59	1.94	0.47	0.21
	Max.	3.56	1.18	2.17	4.65	10.90	44.00	8.33	7.91	6.27	2.97	1.31
	Min.	1.79	0.00	0.00	1.24	1.93	4.33	3.87	2.86	1.96	1.58	0.72
Free cholest., *mg. %*	Mean	43.7	69.9	12.9	51.3	78.3	62.8	24.8	5.8	3.5	3.4	2.7
	S.D.	14.4	48.7	11.6	23.4	21.9	14.3	9.6	4.6	2.9	3.2	2.6
	Max.	71.4	161.0	40.5	91.4	109.9	91.8	40.2	17.4	7.8	10.8	7.6
	Min.	27.4	3.2	0.7	22.5	37.6	36.7	7.7	0.0	0.0	0.0	0.0
Cholest. esters, *mg. %*	Mean	215	161	68	262	415	374	237	163	89	35	13
	S.D.	46	119	50	108	104	72	37	22	40	35	16
	Max.	282	296	165	446	547	548	266	200	151	95	35
	Min.	148	12	0	134	257	268	175	114	12	2	0
P-lipids, *mg. %*	Mean	222	278	79	190	298	303	265	244	231	216	203
	S.D.	32	129	39	63	60	36	29	16	16	21	40
	Max.	280	465	169	318	395	340	296	268	270	355	313
	Min.	170	105	41	117	208	251	192	208	205	190	143
Neut. fat, *mg. %*	Mean	161	749	55	77	98	67	99	90	109	127	138
	S.D.	73	440	25	36	58	43	53	33	56	39	43
	Max.	287	1,684	110	142	197	139	221	148	194	194	223
	Min.	46	148	23	15	32	0	32	15	17	64	67
Total lipids, *mg. %*	Mean	635	1,247	215	579	883	807	626	502	433	382	352
	S.D.	155	678	101	204	228	122	75	48	64	39	53
	Max.	911	2,494	440	900	1,176	1,034	769	589	536	467	460
	Min.	463	326	82	331	543	600	530	432	335	334	279

* On 10 sera. † On 8 sera.

centration at each of the 10 levels sampled indicates the over-all variability attributable to technical errors of centrifugation, sampling and analytical measurement as well as the physiologic variation in concentration of proteins and lipids in a small group of healthy young men. With so many important sources of variability we are of the opinion that the reproducibility of distribution curves for our different components in the normal serum is remarkably high. In general, reproducibility is greater in the lower half of the column. The lowest standard deviation expressed as per cent of the mean concentration is found in the No. 5 sample for free cholesterol, in the No. 7 sample for phospholipids, ester cholesterol and total proteins, in the No. 6 sample for albumin, in one

of the two bottom samples for neutral fat, and globulin. In general, reproducibility of curves of distribution is of the same order as reproducibility of a given measurement for whole serum in descending order: specific gravity, total protein, albumin, phospholipids, globulin, cholesterol ester, free cholesterol and neutral fat. No doubt, the large technical error in estimation of neutral fat contributes to its poor reproducibility.

We have examined our data to determine what portion of the centrifugates contributed most to variation in concentration of the different lipid components in normal whole serum. Samples from any given tube level should contribute to variability of concentration of a given component in whole serum in proportion to the magnitude of the standard deviation of mean content of that component in the sample. The three highest values for any one lipid among the 10 samples were found in either the zone of minimum or of low-medium density.

The most reasonable explanation for the peculiarities in distribution of lipids we are reporting is that they are associated with proteins and that their behavior under high centrifugal forces is influenced by peculiarities of both protein and lipid components and by the quantity and characteristics of non-lipid proteins present in the serum. The role of the protein portion of the complex in determining the localization in the centrifugate seems especially important for those in the lower levels. We believe an important mechanism in the distribution of lipids depends upon the behavior of a lipid complex when surrounded by media of differing density. The sedimentation of proteins results in a gradient of density of the surrounding medium, which varies from specific gravity of about 1.006 at the top to one of about 1.110 at the bottom. Certain lipid complexes, with densities between these limits, tend to become concentrated at their own density level by both flotation from lower levels and sedimentation from higher portions of the column. This concept was first suggested by one of us (J. R. S.) early in 1949 because the peak concentration of lipids in the zone of low-medium density, particularly that of free cholesterol, tended to appear in centrifugate of about the same specific gravity, regardless of length of centrifugation and original specific gravity of the whole serum (2).

Gofman and associates arrived at a similar concept from study of analytical ultracentrifuge patterns. Our observations with different technic and data confirm the basic principle described by the Berkeley workers whose publication (13) preceded ours. Although the importance of the protein density gradient in determining localization of certain lipoproteins in the ultracentrifugate of whole serum is now well established, we still lack accurate expression of the relationship between density of suspending medium and lipid complex at various levels in the column. There is evidence that the specific gravity of the two may differ significantly after four hours of centrifugation, in spite of the tendency toward equality. Certainly at the top of the column the lipid complexes are less dense than the suspending medium. Our study of correlation between specific gravity and total protein concentration of the centrifugate shows the presence of a non-protein solution with specific gravity of 1.006 (37). Furthermore, estimations of concentration of sodium and potassium at various levels gave values within the normal range for whole serum. Our observation that the specific gravity of centrifugate of our top sample is approximately that of water can only be explained by the presence of lipid aggregates having a lower specific gravity than that of the medium in which they are suspended. Lindgren, Elliott and Gofman have reported that chylomicrons and other lipid particles have a specific gravity of less than 1.006 (17). Obviously the two ends of the column represent a special condition where lipid complexes may be stopped while moving toward their own density levels. We have measured the density of lipid complexes in the zone of low-medium density and find their densities to be slightly higher than that of the suspending medium (38).

The possibility remains that the lipid distribution patterns observed might be due in part to accumulations of non-protein lipid aggregates at some levels. That this is not the case, with the possible exception of the cream layer, however, is strongly suggested by the observation that the lipid aggregates with the greatest proportion of the lipid of highest density, cholesterol, occur relatively high in the tube, while those complexes containing the greatest proportions of low density

lipids, neutral fat and phospholipids, occur deep in the tube at high density levels.

Evidence that the described technic for the use of the quantity ultracentrifuge achieves a high degree of differentiation of lipid complexes are: *1*) The individuality of curves of distribution for the different lipid components, and as a consequence extreme variations in different samples in relationships which in normal whole serum tend to great constancy, such as ratios of esterified to free cholesterol, and cholesterol to lipid phosphorus (39). *2*) Marked tendency to constancy of interrelationship among lipid components in each of five top samples. This evidence will be presented in detail later. One of the important methods for differentiation of lipoproteins does not show differences between fractions in the proportions of free and esterified cholesterol (40).

The observation that the four lipid constituents estimated, free and esterified cholesterol, phospholipids and neutral fat, were all found in high concentration slightly above the middle of the column and that in the layers beneath, first free then esterified cholesterol approached or reached zero concentration, although the others remained at high levels, is interpreted as evidence that some serum lipoproteins contain cholesterol in the esterified form only and others are completely lacking in cholesterol. The existence of such lipoproteins, as far as we are aware, has not been previously pointed out. Albrink, Man and Peters, in a recent study of the lipids of whole serum (41), presented mathematical evidence that might be interpreted as indicating the presence of a moiety of lipid phosphorus which is not associated with free cholesterol.

It is entirely possible that better differentiation of lipoproteins may be achieved when the redistribution of large molecules by high centrifugal forces has reached equilibrium than is possible by our usual four hour technic, as is shown in Figure 3. When centrifugation is prolonged, however, the proteins, including the lipoproteins, are forced into such a small fraction of the tube and reach such high concentration there that two difficulties may interfere with exploitation of the possible advantages: accurate collection of thin layers of centrifugate and imperfect reorientation of layers when centrifugation ends.

SUMMARY

1. A procedure for the study of untreated human serum with the quantity ultracentrifuge is described, in which the column of ultracentrifugate is sampled at 10 levels and analyzed by quantitative methods for proteins and lipids.

2. Detailed results of analyses of centrifugate and whole serum of 14 healthy young men are shown in tables, which also indicate over-all variability of concentration due to errors of physical method, sampling, analytical technics, and physiologic variables. In graphs are presented mean curves of distribution in the column of centrifugate of specific gravity, total protein, albumin, globulin, free and esterified cholesterol, phospholipids, and neutral fat. A similar graph shows results of prolonged centrifugation.

3. The principal features of the centrifugate are shown by the characteristics of five zones of increasing density: (*a*) Zone of minimum density at the top of the column; neutral fat is the principal constituent; content highly variable, representing about one-half of whole serum neutral fat. (*b*) Zone of minimum concentration; poor in proteins and lipids; inconstant composition with usual centrifugation. (*c*) Zone of low-medium density, not exceeding that of normal serum; a broad zone centering above the mid-point of the column; moderate increase in concentration over whole serum of all lipid constituents measured, except neutral fat; contains nearly one-half the lipids in the serum. In this zone more than in any other are the lipids, excepting neutral fat, principally responsible for the variation in concentration of such lipids in normal serum. (*d*) Zone of high-medium density, exceeding that of normal serum; contains little free cholesterol, but the esterified form is present in disproportionately great concentration. (*e*) Zone of high density at the bottom, with the highest concentration of proteins; especially rich in globulins, and contains little cholesterol in any form, but with concentrations of phospholipids and neutral fat approximating that of whole serum.

4. The distribution of lipids in the column of centrifugate is greatly influenced by the protein density gradient, the characteristics of which in normal serum depend upon the choice of mean

centrifugal force and time of centrifugation. In the standard procedure described, the density gradient and distribution of proteins and lipids is adapted to present limitations of sampling.

5. The distribution of lipids in the lower portion of the centrifugate shows the presence there of serum lipoproteins containing either phospholipids or neutral fat, or perhaps both; in one type the cholesterol is only of the esterified form, and in the other there is no cholesterol whatever.

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EVIDENCE SUGGESTING RENAL TUBULAR EXCRETION OF POTASSIUM IN MAN DURING RECOVERY FROM ACUTE RENAL INSUFFICIENCY [1]

By JONAS H. SIROTA [2] AND IRVING G. KROOP [3] WITH THE TECHNICAL ASSISTANCE OF HATTIE WARMBRAND

(From the Departments of Medicine and Pediatrics and the Cardiovascular Research Group, The Mount Sinai Hospital, New York, N. Y.)

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In 1942 Winkler and Smith (1), studying the simultaneous renal clearances of exogenous creatinine and potassium in dogs following the injection of potassium salts, concluded that potassium is normally filtered at the glomerulus and subjected to a variable degree of tubular reabsorption. The highest potassium/creatinine clearance ratio obtained by these authors was 0.40. In 1948 Berliner and Kennedy (2), and Mudge, Foulks and Gilman (3) independently and simultaneously published convincing evidence for active renal tubular excretion [4] of potassium in the dog. The former authors obtained potassium/creatinine clearance ratios as high as 1.33 in trained dogs receiving oral potassium chloride for one week before the experiment, and hypertonic solutions of this substance intravenously during the experiment. The latter investigators obtained potassium/creatinine clearance ratios as high as 1.39 in anesthetized dogs receiving intravenous potassium chloride during urea-induced osmotic diuresis. McCance and Widdowson (4) in 1937 were the first to suggest the possibility of tubular excretion of potassium in man. They observed an isolated potassium/inulin clearance ratio of 1.24 in a man suffering from acute alkalosis at a time when his inulin clearance was depressed to 9.3 ml./min. In 1943 Keith, King and Osterberg (5) stated that they observed a potassium/inulin clearance ratio greater than unity in a subject with severe renal insufficiency. No details are given by these authors. Leaf and Camara (6), using 24-hour endogenous "creatinine" clearances as a measure of glomerular filtration rate, obtained potassium/endogenous "creatinine" ratios ranging from 1.21 to 2.07 in four subjects with severe chronic renal insufficiency. Nickel and Bradley (7) have confirmed these observations in chronic renal insufficiency by obtaining potassium/inulin clearance ratios significantly greater than unity.

Berliner (8) suggests that potassium is normally excreted by the triple mechanisms of filtration, tubular reabsorption and tubular excretion. Since the filtered load of potassium is usually much greater than that excreted in the urine it is apparent that demonstration of tubular excretion of this ion in man is dependent upon means for maximally inhibiting tubular reabsorption of potassium by means of osmotic diuresis, as in the dog experiments of Mudge, Foulks and Gilman; by saturating the organism with potassium, as in the dog experiments of Berliner and Kennedy; or by producing a marked reduction of the filtered potassium load. Since the first and second of these methods are too drastic for man, one is necessarily limited to the study of pathologically induced depressions of filtered potassium loads. It occurred to us that the recovery phase of acute renal insufficiency in man would lend itself to such a study, as previously published data (9) indicate that markedly reduced filtration loads may be present at a time when tubular functional integrity is returning. Analysis of our electrolyte and clearance data in five cases of acute renal insufficiency studied by us does in fact strongly suggest that tubular excretion of potassium may occur during the recovery phase.

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[2] Sarah Welt Fellow in Medicine.

[3] Dazian and Sarah Welt Fellow in Medicine. Present address: Jewish Sanitarium and Hospital for Chronic Disease, Brooklyn, New York.

[4] The term "tubular excretion," rather than "tubular secretion," is used advisedly at the suggestion of Dr. Stanley E. Bradley, since the available evidence indicates that the mechanism may be one of ion exchange, akin to the processes of acid and ammonia production by the distal tubule, rather than the processes associated with PAH, diodrast and PSP secretion by the proximal tubule.

METHODS

Simultaneous daily 24-hour renal clearances of endogenous "creatinine" and potassium were determined in five patients recovering from the oliguric phase of acute renal insufficiency caused by carbon tetrachloride poisoning in four and sulfonamide intoxication in one. The clinical details of these cases will be described elsewhere. Simultaneous acute renal clearances of inulin and endogenous "creatinine" were performed at random intervals.

Daily plasma analyses for potassium and "creatinine" were performed upon fasting morning blood specimens drawn without stasis into heparinized tubes and immediately centrifuged. Twenty-four hour urines were collected in bottles containing 3 ml. of toluol and were analyzed for potassium and "creatinine" content. When the urine volumes were under 500 ml./day the patients were catheterized at the end of each 24-hour period to insure complete bladder emptying. The remainder of the 24-hour urines were collected by spontaneous voiding. Indwelling catheters were not used. Bilateral ureteral catheterization was performed on one subject (L. P.) on the day of admission. For the acute inulin clearance determinations indwelling urethral catheters were used and the bladders were emptied by means of water and air washouts. The above constituted the only instances of urological manipulation. Representative plasma concentrations for calculating the 24-hour clearances of potassium and endogenous "creatinine" were obtained by averaging the values of two successive mornings. This average value was then applied to the urine collected during the intervening 24 hours. Inulin clearances were performed according to the standardized techniques of Smith, Goldring and Chasis (10).

Initially the patients' diets consisted of sweetened fluids of low potassium content in volumes calculated to maintain them in slightly negative fluid balance. With the onset of diuresis, they were placed on a low protein, high carbohydrate, low sodium (5 to 10 meq./day), constant potassium (40–50 meq./day) diet as tolerated. When the diuretic phase was well established fluids and salt were added *ad lib.* Regular ward diets were given when evidences of nitrogen retention were minimal.

Inulin was determined by a modification of Harrison's method as described by Goldring and Chasis (11). "Creatinine" was determined in plasma and urine by means of the Bonsnes and Taussky method (12). The plasma proteins for this determination were precipitated by a modification of the Folin-Wu method (13), which utilizes one-half the amount of tungstate usually recommended. When employed with the Bonsnes-Taussky modification of the Jaffe reaction this protein precipitation method yields better plasma recoveries of added creatinine from concentrated precipitates (1 to 4 precipitation dilution) than the standard Folin-Wu technique.[5] The Coleman Jr. Spectrophotometer was used for all colorimetric determinations. Potassium analyses were performed with the Perkin-Elmer internal standard flame photometer Model 52A in accordance with the techniques recommended in the Perkin-Elmer instruction manual. A standard potassium curve was determined with each series of determinations and the closest standard point was checked following each unknown determination. Lithium, at a concentration of 20 meq./L., was used as the internal standard. Utilizing this technique plasma concentrations in normal subjects ranged from 3.7 to 5.0 meq./L. Recoveries of added potassium to plasma and urine ranged from 97.0 to 102 per cent.

RESULTS

Daily urine flows (V), 24-hour endogenous "creatinine" clearances (C_{CR}), plasma potassium concentration (P_K), 24-hour urine potassium output (UV_K), potassium/endogenous "creatinine" clearance ratios (C_K/C_{CR}), occasional endogenous "creatinine"/inulin clearance ratios (C_{CR}/C_{IN}), plasma creatinine concentrations (P_{CR}), and plasma urea nitrogen concentrations (P_U) are presented in Table I for all five subjects. It is evident that during the early recovery phase four of the subjects exhibited 24-hour potassium clearances of greater magnitude than simultaneous endogenous "creatinine" clearances ($C_K/C_{KR} > 1.00$). This phenomenon occurred in the four patients with carbon tetrachloride poisoning and was absent in the one subject with sulfonamide intoxication (L. P.). In subject F. Mc. a C_K/C_{CR} ratio of 2.20 was present on the first day of increased urine volume. True diuresis did not begin until the next day. This ratio continued to be greater than 1.00 for seven additional days, until a C_{CR} of 12.1 ml./min. was attained. In subject H. Z. the C_K/C_{CR} ratio was 1.36 on the first day of observation,

[5] With elevated concentrations of plasma creatinine concentrated precipitates are not necessary. Precipitation dilutions of 1 to 6 or higher may be employed with plasma creatinine concentrations about 2.0 mgm./100 ml. For these more dilute precipitates the standard Folin-Wu tungstic acid method may be used, as recoveries by both methods are equally good at these higher dilutions.

The use of ⅓ molar tungstate with ⅔ molar sulfuric acid as advocated by Brod and Sirota (13) for low precipitation dilutions results in an acid filtrate. The alkaline picrate used to develop the Jaffe color must therefore be sufficiently alkaline to obviate errors which may be introduced by this acid filtrate. The Bonsnes-Taussky modification of the Jaffe reaction was found satisfactory. No differences were observed in the final Jaffe colors developed when this method was used with both the standard (neutral filtrate) and modified (acid filtrate) protein precipitation methods at precipitation dilutions above 1 to 6.

TABLE I

Daily values for urine flows, endogenous "creatinine" clearances, plasma potassium concentrations, urinary excretion of potassium, potassium/endogenous "creatinine" clearance ratios, random endogenous "creatinine"/inulin clearance ratios, and plasma creatinine and urea nitrogen concentrations in five subjects during recovery from acute renal insufficiency *

Day from onset of oliguria	Subject F. Mc.								Subject H. Z.								Subject F. H.							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	V	C_{CR}	P_K	UV_K	$\frac{C_K}{C_{CR}}$	$\frac{C_{CR}}{C_{IN}}$	P_{CR}	P_U	V	C_{CR}	P_K	UV_K	$\frac{C_K}{C_{CR}}$	$\frac{C_{CR}}{C_{IN}}$	P_{CR}	P_U	V	C_{CR}	P_K	UV_K	$\frac{C_K}{C_{CR}}$	$\frac{C_{CR}}{C_{IN}}$	P_{CR}	P_U
4																								
5																								
6																								
7							19.6	83.0															10.0	—
8	240	0.80	6.58	3.51	0.46		22.4	58.0															18.0	119
9	647	0.52	6.75	11.1	2.20		24.3	78.0							28.4	140							16.2	146
10	1,500	0.88	7.00	21.4	2.41		24.6	123	424	0.92	7.75	14.0	1.36		30.7	166	2,290	4.98	3.30	37.3	1.58	1.15	16.6	125
11	2,010	3.79	7.04	42.5	1.11		26.5	128	880	1.60	7.62	22.3	1.27		31.9	138	2,650	4.73	3.26	37.4	1.68	1.11	16.9	166
12	3,000	4.10	7.40	55.8	1.28		26.5	126	1,205	3.00	8.04	40.2	1.16		31.6	145	2,970	4.59	3.67	37.7	1.56	1.06	15.2	103
13	2,970	7.28	6.75	87.6	1.24		25.0	116	1,808	4.20	7.58	42.3	0.92		32.0	189	3,700	8.26	3.82	51.0	1.13		15.0	110
14	2,040	8.60	6.82	99.4	1.18	1.25	20.4	—	1,180	5.05	7.60	26.5	0.48		31.0	226	4,580	10.2	4.48	67.0	1.02		14.1	118
15	2,380	10.6	6.10	122	1.31		17.7	131	970	5.20	7.68	41.8	0.73		26.8	—	4,922	14.4	4.25	84.3	0.96		10.1	98
16	2,450	12.1	6.08	129	1.22		14.6	130	1,296	5.34	7.62	92.2	1.57		21.5	212	4,500	15.7	3.42	80.4	1.04		8.50	101
17	1,440	10.6	5.82	77.0	0.87		11.7	106	1,965	8.62	7.60	110	1.17	1.36	17.2	193	4,000	23.9	3.55	86.4	0.71		6.01	95.0
18	1,291	16.2	5.61	66.4	0.51		9.20	86.0	1,570	10.3	7.58	77.5	0.69		13.8	—	4,500	27.8	3.84	87.7	0.57		4.62	—
19	1,340	18.5	5.43	74.2	0.51		8.30	80.0	1,920	15.8	6.42	—	—		8.60	—	3,800	30.5	—	89.0	—		3.74	66.0
20	1,592	24.0	5.30	—	—		6.35	78.0	1,460	22.4	5.41	—	—		4.84	—	3,030	34.2	4.05	83.5	0.42	1.02	3.05	—
21	1,904	27.3	—	—	—		4.20	64.0	2,799	29.9	4.08	80.8	0.46		3.55	168	3,390	44.5	—	80.3	—		2.96	41.0
22	1,717	33.4	—	—	—		2.86	—							—	—	2,900	55.3	—	55.2	—		2.55	35.0
23	1,830	49.2	4.28	73.4	0.24		2.80	—							—	—		61.2	3.91	41.0	0.12		2.24	24.0
24							—	—							—	—							2.06	—
25							—	31.0							1.40	21.0							1.95	20.0
26						1.20	2.00	29.0							—	—							1.90	24.0
27							—	—							—	—							1.72	17.0
30							1.72	—							—	—							1.70	18.0

Day from onset of oliguria	Subject W. M.†								Subject L. P.‡							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	V	C_{CR}	P_K	UV_K	$\frac{C_K}{C_{CR}}$	$\frac{C_{CR}}{C_{IN}}$	P_{CR}	P_U	V	C_{CR}	P_K	UV_K	$\frac{C_K}{C_{CR}}$	$\frac{C_{CR}}{C_{IN}}$	P_{CR}	P_U
4									78	1.65	10.8	0.45	0.02	1.80	14.5	125
5									228	2.00	10.5	1.42	0.05		23.7	154
6									148	1.13	10.6	1.10	0.06		27.8	135
7									250	1.90	8.80	1.68	0.07		25.6	187
8							15.2	108	530	2.50	8.82	4.24	0.13		26.8	211
9							16.8	108	1,200	5.20	8.81	11.1	0.17		26.6	—
10							18.4	69.0	1,072	5.86	8.30	7.80	0.11		24.4	186
11	95	0.48	7.31	2.84	0.56	0.98	18.6	71.0	2,410	13.0	7.35	25.6	0.19	1.45	18.6	214
12	72	0.43	7.61	2.71	0.58		18.6	73.0	2,535	15.2	7.17	64.0	0.41		13.4	196
13	96	0.57	8.04	3.52	0.53		18.3	86.0	3,170	27.7	6.81	84.2	0.31		8.40	153
14	119	0.68	8.16	6.68	0.84		19.6	92.0	3,380	31.5	6.62	58.6	0.19		5.90	110
15	200	—	8.21	—	—		20.9	98.0	3,800	83.0	5.33	42.6	0.07		3.30	92
16	270	2.65	7.91	25.3	0.84		20.5	96.0	2,660	85.2	5.13	48.5	0.08		2.85	52
17	604	3.04	—	—	—		21.0	92.0	2,620	89.6	4.96	54.0	0.08		2.10	43
18	1,193	3.94	7.96	56.2	1.24		20.7	98.0								
19	2,272	4.06	8.10	84.1	1.77	2.45	20.2	98.0								
20	2,527	7.50	8.03	132	1.52		18.2	91.0								
21	2,653	10.1	7.81	134	1.18		16.1	105								
22	2,652	14.2	6.52	149	1.12		13.2	104								
23	2,249	18.6	6.36	176	1.04		9.80	78.0								
24	2,228	22.5	5.83	151	0.80		8.20	58.0								
25	2,370	25.1	5.43	116	0.59		5.31	68.0								
26	2,378	30.1	5.26	71.5	0.31	1.20	4.83	40.0								
27							4.20	42.0								
30	650	52.7	4.21	30.4	0.10		2.60	38.0								

* The plasma potassium and creatinine concentrations listed represent the average of two successive mornings. The plasma urea nitrogen concentrations represent single values.

† This patient was placed in the "artificial kidney" on the ninth and 15th days from the onset of oliguria.

‡ This subject developed acute renal inaufficiency following sulfonamide administration. There were no sulfonamide crystals demonstrated by renal pelvic lavage.

Column 1. V = urine flow ml./24 hr.
Column 2. C_{CR} = 24-hr. endogenous "creatinine" clearance ml./min.
Column 3. P_K = potassium plasma concentration meq./L.
Column 4. UV_K = urine output of potassium meq./24 hr.
Column 5. C_K/C_{CR} = ratio of potassium clearance to endogenous "creatinine" clearance.
Column 6. C_{CR}/C_{IN} = ratio of endogenous "creatinine" clearance to inulin clearance.
Column 7. P_{CR} = creatinine plasma concentration mgm. 100 ml.
Column 8. P_U = urea nitrogen plasma concentration mgm. 100 ml.

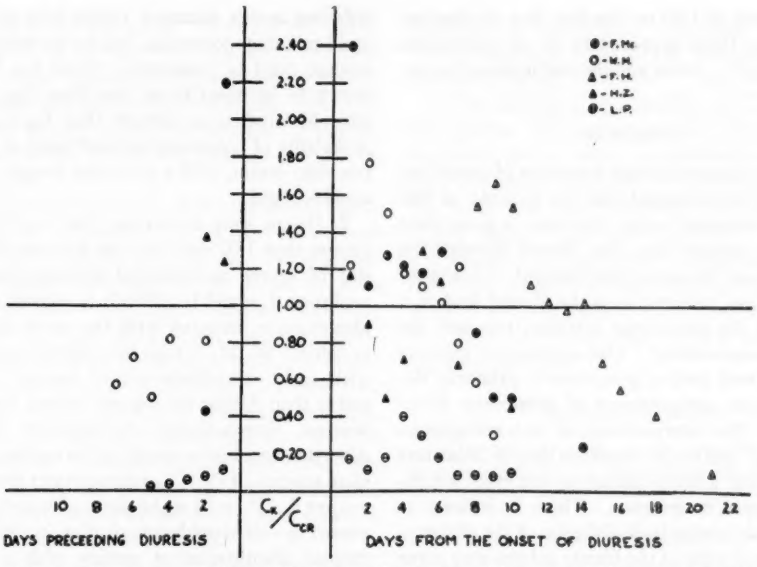

FIG. 1. SIMULTANEOUS 24-HOUR POTASSIUM/ENDOGENOUS "CREATININE" CLEARANCE RATIOS PRESENT IN FIVE SUBJECTS WITH ACUTE RENAL INSUFFICIENCY PRECEDING AND FOLLOWING THE ONSET OF DIURESIS

while still oliguric. For the next seven days it ranged between 0.48 and 1.33, being greater than 1.00 for four of those days and ceasing to be so when C_{CR} reached 10.3 ml./min. Subject F. H. was not oliguric when first observed.[6] The C_K/C_{CR} ratio was significantly greater than 1.00 during the first four days of observation (1.13 to 1.68) and dropped to levels significantly less than 1.00 when C_{CR} reached 23.9 ml./min. Subject W. M. had severe oliguria for 17 days. A C_K/C_{CR} ratio of 1.24 was present on the first day of diuresis. This ratio remained significantly greater than 1.00 for five days. At a C_{CR} of 22.5 ml./min. the C_K/C_{CR} ratio became less than 1.00. Subject L. P. (sulfonamide poisoning) had C_K/C_{CR} ratios well under 1.00 throughout the observed recovery period. The highest ratio attained was 0.41, on the fourth day of diuresis, at a time when C_{CR} was 15.2 ml./min. In all subjects random inulin clearances (C_{IN}) were either similar to or significantly lower than simultaneous endogenous "creatinine" clearances. At no time was C_{IN} significantly higher, suggesting that the 24-hour C_{CR} is at least at the level of C_{IN}, and probably significantly higher most of the time during the early recovery phase. Figure 1 presents all the C_K/C_{CR} ratios plotted against days preceding and following the onset of diuresis. A total of 25 C_K/C_{CR} ratios greater than 1.00 were observed during the time interval representing the period from two days before the onset of diuresis ($V > 900$ ml./day) to 14 days after. The three observed before the onset of diuresis were during increasing urine flows, which had not yet attained the arbitrarily chosen value of 900 ml./day.

The plasma potassium concentrations were elevated in all subjects except F. H. There appeared to be no correlation between the plasma potassium level and the C_K/C_{CR} ratios. Thus, subject L. P. with a plasma concentration of 8.81 meq./L. at the onset of diuresis maintained C_K/C_{CR} ratios well under 1.00; whereas F. H. with a plasma potassium concentration of 3.30 meq./L. revealed a

[6] There was a two-day period of questionable oliguria in this patient following exposure to carbon tetrachloride. When first seen, 10 days after exposure, his blood urea nitrogen was 146 mgm./100 ml. and his plasma "creatinine" concentration was 16 mgm./100 ml. in spite of urine flows of over 2,000 ml./day.

C_K/C_{CR} ratio of 1.58 on the first day of observation. Also, there appeared to be no correlation between C_K/C_{CR} ratios and the oral intake of potassium.

DISCUSSION

In order to prove tubular excretion of potassium it must be demonstrated that the quantity of this substance excreted in the urine over a given time interval is greater than that filtered through the glomeruli over the same time interval. This latter determination, referred to as the filtered load, is a product of the glomerular filtration rate and the plasma concentration.[7] The accuracy of measuring the filtered load of potassium is primarily dependent upon measurement of glomerular filtration rate. The interpretation of both endogenous "creatinine" and inulin clearance data as indicators of glomerular activity during severe renal insufficiency is open to question. There is evidence to suggest that tubular back-diffusion of the glomerular filtrate or some of the filtrate solutes may occur following severe renal injuries (14–17). Under such circumstances the renal clearances of endogenous "creatinine" and inulin may be considerably lower than the true glomerular filtration rate, and hence the filtered potassium load calculated from these data may be significantly lower than the true values. In spite of this cogent objection to our data, we feel that they strongly suggest the occurrence of tubular excretion of potassium for the following reasons:

1. Although the product of C_{IN} or $C_{CR} \times P_K$ may be meaningless under these circumstances because of back-diffusion, the ratio C_K/C_{CR} greater than 1.00 is not. This latter expression simply means that more of the plasma reaching the kidney is being virtually cleared of potassium than of "creatinine." Unless one assumes that the glomeruli have become more permeable to potassium than to "creatinine" or that "creatinine" is back-diffusing across damaged tubule cells at a more rapid rate than potassium, tubular excretion of potassium must be postulated. Since C_{IN} has been shown to be equal to or less than C_{CR}, C_K/C_{IN} must be equal to or greater than C_K/C_{CR}. The probability of potassium back-diffusing at a slower rate than inulin, with a molecular weight of 5,000, appears slight.

2. If one were to assume that C_K/C_{CR} ratios greater than 1.00 were in some way merely the result of severe architectural derangement of the nephrons it would be difficult to explain why this phenomenon occurred with the onset of diuresis in subject W. M., a time thought to be associated with early reestablishment of tubular integrity, rather than during the oliguric period, the time of severest morphological derangement (9, 18).[8] Also, this assumption would fail to explain the complete absence of C_K/C_{CR} ratios greater than 1.00 in subject L. P. with eight days of severe oliguria caused by sulfonamide intoxication in which severe tubular disorganization occurs with a severity equal to other causes of "lower nephron nephrosis" (18).

The disappearance of C_K/C_{CR} ratios greater than 1.00 with rising C_{CR} suggests that the tubular excretion of potassium does not necessarily cease, but might become masked by larger filtered potassium loads available for reabsorption and tubular rejection. We have no explanation for the failure to observe C_K/C_{CR} ratios greater than 1.00 in the one case of sulfonamide intoxication. The nature of the cellular injuries in this case may have been such as to destroy the mechanism responsible for tubular excretion of potassium; or perhaps this phenomenon may indeed be a peculiarity of carbon tetrachloride poisoning. Further observations on subjects with acute renal insufficiency of varied etiology are required to determine this point. However, similar observations have been made which suggest tubular excretion of potassium during acute experimental reductions

[7] In order to calculate the amount of potassium filtered from the product GFR × P_K, one should for greatest accuracy take into account the correction factors for plasma water and the Donnan equilibrium. However, since these two factors are of approximately the same magnitude and apply corrections in opposite directions they may be ignored, since our best methods for measuring GFR have inherent errors of 5 per cent or more.

[8] The onset of diuresis following acute renal insufficiency in the authors' opinion represents reestablishment of tubular functional integrity with respect to the passive back-diffusion of water, as the urine output may increase many fold with little or no change in C_{CR} or C_{IN}. This stage correlates morphologically with regeneration of tubule cells.

of filtered potassium loads. Thus Farber, Alexander and Earle have demonstrated transient C_K/C_{CR} ratios significantly greater than 1.00 following acute experimental anuria in dogs (19), and Breed and Baxter have demonstrated C_K/C_{IN} ratios greater than 1.00 during shock in one human subject (20).

It is probable that some of the potassium found in the urine represents that liberated as a result of renal parenchymal destruction. The quantity of potassium contributed to the urine by such tissue destruction is difficult to assess. However, as Lucké (18) has shown, at the time when diuresis usually occurs, regenerative processes rather than destructive ones are manifest. It would thus be difficult to explain excess potassium excretion on the basis of tissue destruction as late as the 14th day of diuresis. Since urological manipulation was minimal during the periods of study mechanical tissue trauma cannot be implicated as a spurious source of potassium in the urine.

According to Berliner (8) the most potent stimulus for increasing tubular excretion of potassium appears to be high intracellular potassium concentrations. This remarkable tubular adaptability of even the severely damaged kidneys of terminal glomerulonephritis and acute renal insufficiency to rid the organism of excess potassium in spite of markedly reduced glomerular function explains the usual absence of significant hyperkalemia in these conditions so long as urine flow is maintained above the oliguric level.

CONCLUSIONS

1. Simultaneous 24-hour clearances of endogenous "creatinine" and potassium were determined daily in five subjects with acute renal insufficiency resulting from carbon tetrachloride poisoning in four and sulfonamide poisoning in one. Potassium/endogenous "creatinine" clearance (C_K/C_{CR}) ratios significantly greater than 1.00 were found in the four patients poisoned by carbon tetrachloride during early recovery from oliguria, suggesting the occurrence of renal tubular excretion of potassium.

2. A total of 25 C_K/C_{CR} ratios greater than unity were observed with a range of 1.02 to 2.41 and a mean of 1.36. Potassium clearances in excess of simultaneous endogenous "creatinine" clearances were observed as long as the 14th day from the onset of diuresis.

3. The highest C_{CR} at which the C_K/C_{CR} ratio was greater than 1.00 was 18.6 ml./min. It is suggested that in order to demonstrate the presence of tubular excretion of potassium in man, one must seek this phenomenon in the presence of low filtration loads of potassium.

4. Random simultaneous endogenous "creatinine" and inulin clearances during the period of study in these subjects revealed the C_{CR} values to be equal to or greater than the C_{IN} values; never significantly lower.

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We are indebted to Dr. Irving Graef for allowing us to study patient W. M., who was admitted to The Mount Sinai Hospital for the "artificial kidney."

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ELECTROLYTE EQUILIBRIUM DURING MERCURIAL DIURESIS [1, 2]

By WILLIAM B. SCHWARTZ [3] AND WILLIAM M. WALLACE

(From the Medical Services of the Peter Bent Brigham Hospital and the Children's Medical Center and the Departments of Medicine and Pediatrics, Harvard Medical School, Boston, Mass.)

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INTRODUCTION

Earlier workers have shown that the amount of fixed cation contained in the extra urine voided during the course of mercurial diuresis corresponded to that contained in an equivalent amount of body fluid, while the quantity of chloride was greater than that which would be expected to be found in the fluid lost. This differential loss of chloride was shown to result in hypochloremic alkalosis. It was further observed that the administration of acidifying salts prevented the occurrence of alkalosis and at the same time enhanced diuresis (1, 2).

The observations reported in this communication indicate that the alteration in the concentration of chloride and bicarbonate in the serum that follows repeated diureses with a mercurial is usually accompanied by unresponsiveness to further administration of the drug. The various patterns of electrolyte loss observed during mercurial diuresis, their significance in relation to body composition, and their relation to certain clinical and physiologic problems will be discussed.

METHODS AND CALCULATIONS

Ten patients in congestive heart failure as the result of rheumatic or hypertensive heart disease were studied on the metabolism ward of the Peter Bent Brigham Hospital. All were chosen on the basis of their willingness and ability to cooperate in the collection of specimens and the consumption of a constant diet. Six were males and four were females. Control periods were obtained when the clinical situation allowed. In most instances, however, it was deemed necessary to begin therapy at once. Each patient was placed on a constant weighed diet that contained minimal sodium and approximated the usual ward diet with regard to nitrogen and caloric value. Every third day a duplicate diet was analyzed for sodium, chloride, potassium, and nitrogen. All food refused was saved for analysis and the intake figures corrected accordingly. Water intakes were kept as constant as possible.

The mercurial compound (Mercuhydrin) was administered intramuscularly in a standard dose of 2 cc. at the beginning of each day of treatment.

The urine was collected for 24-hour periods, with thymol in chloroform used as preservative. The feces were collected from eight of the ten patients and were pooled in four to six-day periods. Carmine was used to mark the beginning and end of each period. The urine and stools were analyzed for sodium, chloride, potassium, and nitrogen. In addition, titratable acidity, pH, ammonia, and total phosphorus were determined in the urine.

Venous blood was drawn without stasis in oiled syringes at the beginning of each daily collection period while the patient was fasting. At the time of blood sampling the patient was weighed to the nearest 50 grams.

The methods used for determination of sodium, potassium, chloride, nitrogen, pH, and carbon dioxide have been described in a previous paper from this laboratory (3). Urine titratable acidity was determined by the method of Henderson and Palmer (4) and ammonia was determined by the method of Folin (5).

Balance data were calculated as net intake, minus combined output in urine and stool. The quantity of substance in the pooled stool collections was divided by the number of days in the collection period to obtain the average figure for the daily stool output. No unusually large stools were passed by any of the subjects reported in this study and daily losses were assumed to be equal. Changes in the volume of the chloride space and shifts of sodium into and out of the intracellular fluid were calculated by methods previously described and used by various authors, (6–8). The form of the calculation and the factors used have been detailed in a previous paper from this laboratory (3). In calculation of the present data the changes in chloride space were calculated backward from the balance of chloride using the assumption that chloride was distributed through 20% of the body weight at the end of the period of diuresis.

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[2] Presented in abstract form at the 42nd Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., May 1, 1950.

[3] Present address: New England Center Hospital and Tufts College Medical School, Boston, Mass.

RESULTS

The ten patients with congestive heart failure were observed for a combined total of 22 periods during which Mercuhydrin was administered. The essential features with regard to the ratio of sodium to chloride (Na/Cl ratio) of the negative balance, the potassium loss or absence thereof, and the development of unresponsiveness to the diuretic are summarized in Table I. A patient was considered to be unresponsive if he was still edematous and his weight did not change after administration of the diuretic. The data are too few to allow a categorical statement about the relationship of the character of the balance losses to the development of unresponsiveness but a certain grouping of patients appears possible. A review of therapy employed prior to the period of study with respect to diuretics, digitalis and salt restriction showed no apparent correlation with the urine Na/Cl ratio during diuresis. Two of the patients (V. S., B. C.) responded to the diuretic by the loss of body fluid the composition of which was similar to that of extracellular fluid. In these, the Na/Cl ratio of the negative balance was above unity; neither alkalosis nor negative potassium balance developed and the patients were continually responsive until approximately normal weight was reached. In eight of the patients the Na/Cl ratio of the negative balance was less than 1. In these subjects there was a fall in serum chloride and a rise in serum bicarbonate concentrations, often accompanied by a negative balance of potassium and a reduction in serum potassium concentration. In all but one (S. S.) of these patients unresponsiveness appeared. The administration of ammonium chloride in amounts sufficient to return the serum chloride and bicarbonate to normal in the refractory alkalotic patients also restored responsiveness in every instance.

Analytical data from four representative subjects (V. S., F. C., N. B., and S. S.) are presented in Tables II–V and Figures 1–6. Figures 1–4 show the daily values for the serum electrolytes, the course of the cumulative balance of electrolytes, the cumulative course of the change of body weight, and an estimate of the shifts of sodium and potassium between extracellular and intracellular fluid. The cumulative nitrogen balance is plotted as grams times 2.7 to indicate the degree of deviation of potassium and nitrogen from the theoretical losses that would occur if body tissue as a whole were being lost or gained (9). The ordinate indicating the cumulative weight loss is so chosen that one kg. equals a value on the mEq. ordinate equivalent to the mean calculated value for extracellular fluid sodium during the period of observation.

In the theoretical situation in which only extracellular fluid is being lost these two cumulative balances should coincide. Deviation of the two curves from each other indicates unmeasured losses through the skin, losses of intracellular fluid, or shifts of sodium into or out of the intracellular compartment. Minimal sweating occurred during these experiments so that losses of sodium through the skin were probably about 5 to 10 mEq./day (10, 11).

The calculated shifts of sodium between the ex-

TABLE I

Interrelationships of Na/Cl ratio of balance to K loss and unresponsiveness

Subject	$\frac{Na}{Cl}$ of balance			K loss			Remarks
	Diuretic period			Diuretic period			
	1	2	3	1	2	3	
V. S.	1.21	1.33	1.14	0	0	0	Continually responsive
B. C.	1.39			0			Continually responsive
F. C.	.73	.51	.53	+	+	+	Refractory at the end of each period
N. B.	.66	.50		+	+		Refractory at the end of each period
S. S.	.44	.57		+	0		Continually responsive
J. C.	.29	.78	.56	+	0	0	Refractory at the end of each period
C. A	.62	.90		0	0		Refractory at the end of each period
S. H.	.94	.57		0	0		Refractory at the end of each period
J. B.	.60	.61		+	+		Refractory at the end of each period
C. G.	.91	.68		0	0		Refractory at the end of each period

TABLE II

Analytical data and body weights

Patient V. S. PBBH No. 6B120

Day	Medications	Body weight	Intake					Urine									Stool				Serum					
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl	Urea N
		kg.	*cc.*	*m.Eq.*	*m.Eq.*	*m.Eq.*	*gms.*	*cc.*	*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*mM./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*mgm. %*
1	Mercuhydrin 2 cc.	116.65	1700	10	25	14	7.2	5340	534	27	513	15	53	5	9.1	6.8	2	4	1	0.7	7.41	31.0	136	4.0	96.0	12
2	Mercuhydrin 2 cc.	112.15	1700	10	25	14	7.2	6010	607	33	520	10	47	0	13.2	7.2	2	4	1	0.7	7.41	36.5	136	4.1	93.5	9
3	Mercuhydrin 2 cc.	107.65	1700	10	25	14	7.2	4420	451	21	305	10	32	0	11.6	7.6	2	4	1	0.7	7.43	37.5	139	3.5	92.5	
4		104.70	1260	10	25	14	7.2	1735	153	21	76	7	19	0	5.0	7.5	2	4	1	0.7	7.39	36.5	136	4.5	92.0	6
5		104.15	1700	9	18	10	5.5	1500	50	22	21	9	34	1	9.2	6.8	2	4	1	0.7	7.41	34.0	136	4.8	92.5	
6	Mercuhydrin 2 cc.	103.40	1700	10	25	14	7.2	3690	292	30	231	11	43	1	10.3	7.0	1	4	1	0.7	7.43	36.5	135	4.5	92.0	
7	Mercuhydrin 2 cc.	101.45	1675	10	25	14	7.2	3360	262	24	197	11	29	0	6.7	7.4	1	4	1	0.7	7.46	37.0	135	5.0	92.0	
8	Mercuhydrin 2 cc.	99.30	1700	10	25	14	7.2	3300	274	23	224	9	38	0	6.6	7.3	1	4	1	0.7	7.43	33.0		4.0	94.0	10
9	Mercuhydrin 2 cc.	97.60	1700	10	25	14	7.2	4640	422	32	340	10	33	0	7.9	7.6	1	4	1	0.7	7.44	36.0	142	4.0	90.0	
10	Mercuhydrin 2 cc.	94.60	1260	10	25	14	7.2	3360	323	30	232	10	26	0	7.1	7.7	1	4	1	0.7		35.0	135	4.5	92.5	
11	Mercuhydrin 2 cc.	91.65	1700	10	25	14	7.2	3640	313	33	233	13	17	0	7.6	7.5	2	8	1	0.9	7.50	35.5	140	3.8	92.5	
12		89.60	1700	10	25	14	7.2	1940	62	29	47	9	*	1	6.1	6.9	2	8	1	0.9	7.46	36.5		4.3	92.0	
13		88.60	1700	10	25	14	7.2	2100	57	28	23	13	24	0	7.2	7.1	2	8	1	0.9			138	4.3	95.5	
14		87.90	1700	9	15	11	4.5	1170	27	19	15	9	21	5	6.0	6.9	2	8	1	0.9	7.39	36.0		3.6	90.0	
15	Mercuhydrin 2 cc.	88.05	1625	10	25	14	7.2	2920	164	22	155	10	25	16	8.8	6.6	2	8	1	0.9	7.39	33.5	136	4.0	91.5	10
16	Mercuhydrin 2 cc.	86.05	1700	10	25	14	7.2	2410	125	18	112	10	23	11	8.2	6.6	2	8	1	0.9	7.38	36.0		3.6	91.5	
17	Mercuhydrin 2 cc.	85.00	1700	10	25	14	7.2	2040	96	18	87	11	18	10	7.6	6.4	1	6	1	0.8	7.42	35.0	138	3.6	90.0	
18		84.45	1700	10	25	14	7.2	1120	22	16	14	10	18	11	5.4	5.8	1	6	1	0.8	7.38	35.0		3.8	89.0	
19		84.60	1700	10	25	14	7.2	1450	17	19	8	12	24	13	7.4	5.9	1	6	1	0.8	7.42	34.0		4.3	87.0	28
20		84.30	1700	10	25	14	7.2	1470	13	16	4	12	22	17	7.4	5.5	1	6	1	0.8			140	4.8	87.0	
21		84.30	1700	10	25	14	7.2	1650	15	20	8	12	22	14	8.4	5.6	1	6	1	0.8			136	4.0	90.0	
22		83.80	1700	10	25	14	7.2	1330	13	16	5	8	20	18	6.1	5.2	1	6	1	0.8	7.42	34.0	136	4.0	87.0	38
23		83.80																								

* Unsatisfactory determination.

TABLE III

Analytical data and body weights

Patient F. C. PBBH No. 8A48

Day	Medications	Body weight	Intake					Urine									Stool				Serum					
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl	Urea N
		kg.	*cc.*	*m.Eq.*	*m.Eq.*	*m.Eq.*	*gms.*	*cc.*	*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*mM./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*mgm. %*
1		57.00	2500	20	58	27	12.5	2000	1	38	43	11	41	18	11.2	5.9	1	28	2	1.2	7.36	24.5	143	6.4	102.0	9
2	Mercuhydrin, 2 cc.	56.70	2500	20	58	27	12.5	5360	354	123	473	21	54	38	13.2	5.0	1	28	2	1.2	7.39	26.0	141	5.8	102.5	
3	Mercuhydrin, 2 cc.	53.65	2500	20	58	27	12.5	2100	84	55	111	17	35	26	6.9	5.6	1	28	2	1.2	7.43	31.0		4.8	99.0	
4	Mercuhydrin, 2 cc.	53.65	2500	20	58	27	12.5	2670	27	48	51	16	35	19	8.4	5.8	1	28	2	1.2	7.42	32.5	144	5.3	98.5	10
5	Mercuhydrin, 2 cc.	53.45	2500	20	58	27	12.5	2360	35	59	50	14	29	19	7.8	6.2	1	18	1	1.2	7.42	34.5	145	5.5	97.0	
6		53.80	2500	20	58	27	12.5	2640	8	42	21	16	28	21	6.4	5.9	1	18	1	1.2		34.5	142	4.7	97.0	
7	HCl, 80 m.Eq.*	53.70	2500	20	58	107	12.5	1620	2	34	23	17	27	24	7.0	5.6	1	18	1	1.2	7.41	34.0	142	5.6	99.5	
8	HCl, 84 m.Eq.*	54.40	2500	20	58	111	12.5	1580	2	40	33	21	39	25	8.0	5.7	1	18	1	1.2	7.40	31.0	141	6.2	103.0	
9	HCl, 84 m.Eq.*	55.75	2500	20	58	111	12.5	2860	4	74	86	21	58	30	10.6	5.6	1	11	1	0.9	7.37	26.0	141	6.6	102.0	
10	Mercuhydrin, 2 cc.	54.75	2500	20	58	27	12.5	4280	253	146	409	29	62	44	16.3	5.0	1	11	1	0.9	7.36	26.5	143	4.8	106.0	
11	Mercuhydrin, 2 cc.	52.85	2500	20	58	27	12.5	2140	44	53	114	19	44	34	7.0	5.7	1	11	1	0.9	7.41	30.0	144	4.8	102.0	
12	Mercuhydrin, 2 cc.	52.60	2500	20	58	27	12.5	2580	40	52	109	14	†	†	9.0	†	1	11	1	0.9	7.43	31.5	140	4.8	98.5	
13	HCl, 84 m.Eq.*	52.30	1950	20	58	111	12.5	1940	4	18	26	21	54	19	8.7	6.0	1	15	1	1.0		32.0	142	4.3	99.5	
14	HCl, 84 m.Eq.*	52.35	2500	20	58	111	12.5	1600	5	14	24	14	46	15	7.9	6.0	1	15	1	1.0	7.38	29.5	141	5.0	104.0	
15	HCl, 84 m.Eq.*	53.35	2500	20	58	111	12.5	1110	3	52	71	17	39	21	8.3	5.5	1	15	1	1.0	7.38	28.0	143	6.0	105.5	
16	Mercuhydrin, 2 cc. HCl, 84 m.Eq.*	54.60	2500	20	58	111	12.5	4145	102	129	270	28	72	87	8.3	5.0	1	15	1	1.0	7.35	26.0	139	5.8	106.5	
17	Mercuhydrin, 2 cc. HCl, 21 m.Eq.*	52.40	2500	20	58	48	12.5	2500	109	93	213	27	61	38	8.8	5.2	1	15	1	1.0	7.37	29.0	140	4.5	103.5	
18	HCl, 84 m.Eq.*	51.55	2500	20	58	111	12.5	2200	62	63	134	19	‡	31	8.8	5.3	1	15	1	1.0	7.40	29.5	140	4.0	103.5	
19		51.45																			7.39	28.5	141	4.2	107.5	

* Chloride administered as HCl is included in total intake value.

† Specimen deteriorated before analysis.

‡ Specimen lost.

TABLE IV

Analytical data and body weights

Patient N. B. PBBH No. 9A140

Day	Medications	Body weight	Intake					Urine									Stool				Serum					
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl	Urea N
		kg.	*cc.*	*m.Eq.*	*m.Eq.*	*m.Eq.*	*gms.*	*cc.*	*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*mM./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*mgm. %*
1		48.40	1500	7	57	12	6.8	1160	1	36	4	10	3	4	4.4	7.0	1	6	1	0.9		32.5	144	4.8	102.5	8
2	Mercuhydrin, 2 cc.	48.50	1500	7	57	12	6.8	2250	126	124	214	14	6	10	6.0	6.5	1	6	1	0.9	7.41	33.0	144	4.8	100.5	
3	Mercuhydrin, 2 cc.	47.35	1500	7	57	12	6.8	2410	150	78	213	12	6	9	5.3	6.4	1	6	1	0.9	7.40	35.0	145	3.3	95.5	
4	Mercuhydrin, 2 cc.	46.40	1500	7	57	12	6.8	1635	41	57	56	13	3	3	4.7	6.9	1	6	1	0.9		35.5	144	3.3	95.0	
5		46.00	1500	7	57	12	6.8	1380	5	48	1	14	5	2	5.9	7.1	1	6	1	0.9	7.51	36.5	144	3.5	92.5	
6	NH_4Cl, 112 m.Eq.*	45.90	1500	7	57	124	8.4	1400	2	34	1	10	5	2	6.3	6.9	1	4	1	1.3	7.53	40.0	144	4.0	92.0	
7	NH_4Cl, 112 m.Eq.*	45.70	1500	7	57	124	8.4	1000	1	19	1	4	27	7	7.3	6.7	1	4	1	1.3	7.48	36.5	146	4.5	98.5	
8	NH_4Cl, 112 m.Eq.*	46.15	1340	4	35	119	5.7	910	1	14	3	8	29	9	8.0	6.5	1	4	1	1.3	7.43	32.5	143	4.3	102.0	11
9	NH_4Cl, 75 m.Eq.*	46.15	1500	7	57	87	7.9	920	2	22	18	3	27	7	7.6	6.4	1	4	1	1.3	7.41	25.0	143	4.5	109.0	
10		46.45	1500	7	57	12	6.8	1330	3	47	43	4	43	12	8.2	†	1	4	1	1.3	7.40	27.0	144	4.8	108.0	
11		46.40	1500	7	57	12	6.8	1265	3	43	34	5	35	14	6.9	6.5	1	6	1	0.7	7.40	26.0	142	5.2	104.5	
12	Mercuhydrin, 2 cc.	46.55	1500	7	57	12	6.8	2730	124	113	238	5	26	12	5.5	6.2	1	6	1	0.7	7.44	27.0	140	4.8	103.5	
13	Mercuhydrin, 2 cc.	45.15	1500	7	57	12	6.8	2600	98	142	232	8	25	13	5.7	6.2	1	6	1	0.7		34.5	142	3.8	97.5	6
14	Mercuhydrin, 2 cc.	44.10	1500	7	57	12	6.8	1640	33	58	49	10	14	14	5.7	6.6	1	6	1	0.7	7.47	36.0	139	3.3	93.5	
15	Mercuhydrin, 2 cc.	43.70	1500	7	57	12	6.8	1340	20	48	27	8	16	†	5.1	6.6	1	6	1	0.7	7.51	39.0	140	3.5	92.5	
16		43.70																								

* Chloride and nitrogen administered as ammonium chloride included in total intake value.

† Unsatisfactory determination.

TABLE V

Analytical data and body weights

Patient S. S. PBBH No. L5455

Day	Medications	Body weight	Intake					Urine									Stool				Serum					
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl	NPN
		kg.	*cc.*	*m.Eq.*	*m.Eq.*	*m.Eq.*	*gms.*	*cc.*	*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*mM./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./day*	*m.Eq./day*	*m.Eq./day*	*gms./day*		*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*m.Eq./l.*	*mgm. %*
1		58.35	1430	16	30	20	5.2	455	1	29	38	8	29	9	5.2	5.3	1	6	3	.6	7.41	29.5	140	4.8	104.5	38
2		57.80	1370	16	30	20	5.2	540	1	32	48	12	37	12	6.4	5.4	1	6	3	.6	7.41	30.5			103.0	
3	Mercuhydrin, 2 cc.	57.65	1500	16	30	20	5.2	2230	143	105	280	15	49	19	5.9	5.3	1	6	3	.6	7.42	30.5	140	5.2	102.5	36
4	Mercuhydrin, 2 cc.	55.40	1450	12	30	14	2.4	1750	91	89	216	21	70	20	6.0	5.1	1	6	3	.6	7.42	33.5	142	3.2	98.5	
5	Mercuhydrin, 2 cc.	54.60	1500	15	21	16	4.3	1550	87	57	184	25	60	26	5.6	5.3	1	6	3	.6	7.45	37.0	144	2.9	95.0	29
6	NH_4Cl I.V.,* 319 m.Eq.	54.40	1840	1	10	329	4.8	930	18	14	47	29	73	25	7.0	6.1	1	6	3	.6	7.47	38.5	154	2.5	86.5	32
7	Mercuhydrin, 2 cc.	54.10	1290	12	21	16	2.6	2340	131	39	210	11	87	15	6.8	5.2	6†	9†	17†	1.1†	7.46	33.5	140	3.5	96.0	42
8	Mercuhydrin, 2 cc.	52.10	1500	13	20	15	5.7	1660	116	36	208	9	83	14	7.3	5.3	1	2	2	.3	7.47	36.0	144	2.8	92.0	31
9	Mercuhydrin, 2 cc.	51.50	1200	13	20	15	5.7	1370	88	24	142	11	57	11	5.4	5.7	1	2	2	.3	7.48	41.5	144	2.6	85.0	
10	Mercuhydrin, 2 cc.	50.85	1500	13	20	15	5.7	1420	75	24	122	12	47	11	5.0	6.0	1	2	2	.3	7.47	44.0	139	2.8	81.5	29
11		50.40	1350	13	20	15	5.7	530	1	8	7	13	37	13	4.7	6.1	1	2	2	.3	7.49	46.5	138	3.3	77.5	32
12		50.60	1025	8	11	10	3.9	330	1	9	4	18	45		3.7	6.3	1	2	2	.3	7.42	44.0	136	3.2	76.5	31
13		51.75																			7.48	42.5	132	4.0	71.0	36

* Chloride and nitrogen administered as ammonium chloride included in total intake value.

† Urine mixed with stool.

tra- and intracellular compartments, as well as the loss or gain of intracellular potassium, are indicated at the bottom of the chart. These intracellular balances are cumulative only for the period indicated. For example, in Figure 3, the shifts during the first four days are indicated at the end of the fourth day; the shift on day 11 is cumulative for days five through 11, and not for the entire 11-day period.

Graphic presentation of the intracellular shifts has been made in terms of the percentage of estimated total extracellular fluid sodium on the day

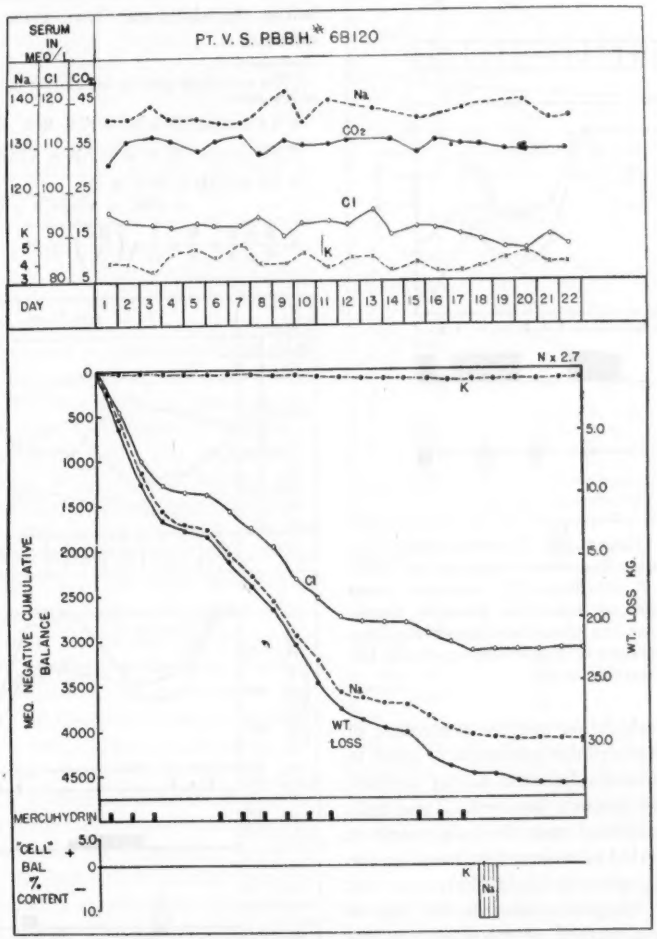

FIG. 1. SERUM ELECTROLYTE CONCENTRATIONS, EXTERNAL AND INTERNAL BALANCES IN RELATION TO THERAPY FOR PATIENT V. S. (TABLE II) SHOWING CONTINUED RESPONSE TO THE DIURETIC WITHOUT PROGRESSIVE CHANGES IN SERUM CONCENTRATIONS

In this and the succeeding three charts the coordinate indicating weight loss is chosen to predict the theoretical sodium loss as calculated from mean extracellular fluid sodium concentration (see text).

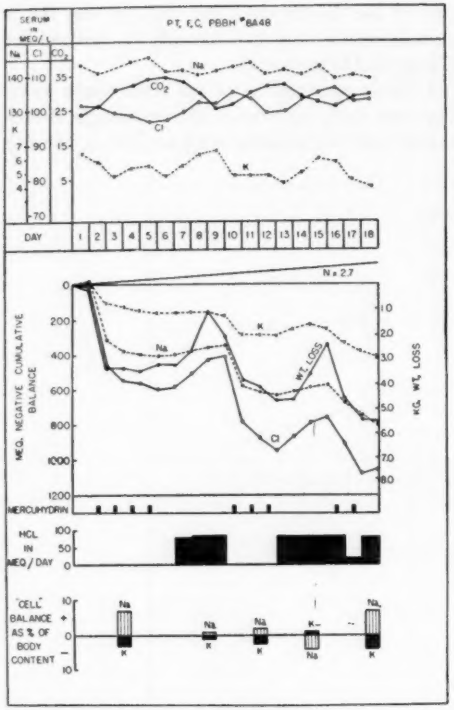

FIG. 2. SERUM ELECTROLYTE CONCENTRATIONS, EXTERNAL AND INTERNAL BALANCES IN RELATION TO THERAPY FOR PATIENT F. C. (TABLE III) SHOWING THREE SUCCESSIVE EPISODES OF MERCURIAL DIURESIS ACCOMPANIED BY ALKALOSIS AND UNRESPONSIVENESS AND DEMONSTRATING THE RETURN OF RESPONSIVENESS WITH HYDROCHLORIC ACID ADMINISTRATION

chosen for the calculation and the percentage of total estimated intracellular potassium in order to bring these calculated values into proper perspective with the total amounts involved. Total body potassium was estimated from the body weight at the end of the period of observation, based on the value 68 mEq./kg. given by Shohl (12).

The necessity for presentation in this way is obvious when the method of calculation is examined. It is evident that analytical errors in the determination of serum sodium and chloride concentrations can produce large changes in the apparent quantity of sodium shifting between cells and extracellular fluid. In the present study the analyses for serum sodium and chloride are assumed to carry a possible error of ± 2%, the technique of the balance, an error of ± 5%, and the determination of weight, an error of ± 1%. When the analytical values are applied in the equations used, calculation of the summation of these possible errors [3] indicates that at least 6% of the extracellular sodium must shift into or out of cells before the shifts can have even statistical significance.

[3] The equations used in calculation of the summation of errors were:

1. $(A \pm a) \pm (B \pm b) = A + B \pm \sqrt{a^2 + b^2}$,
2. $(A \pm a) \times (B \pm b) = AB \pm \sqrt{(AB)^2 + (Ba)^2}$,
3. $(A \pm a)(B \pm b)(C \pm c) = ABC \pm \sqrt{(aBC)^2 + (bAC)^2 + (cAB)^2}$,
4. $\frac{B \pm b}{A \pm a} = \frac{B}{A} \pm \frac{1}{A}\sqrt{\left(\frac{Ba}{A}\right)^2 + b^2}$.

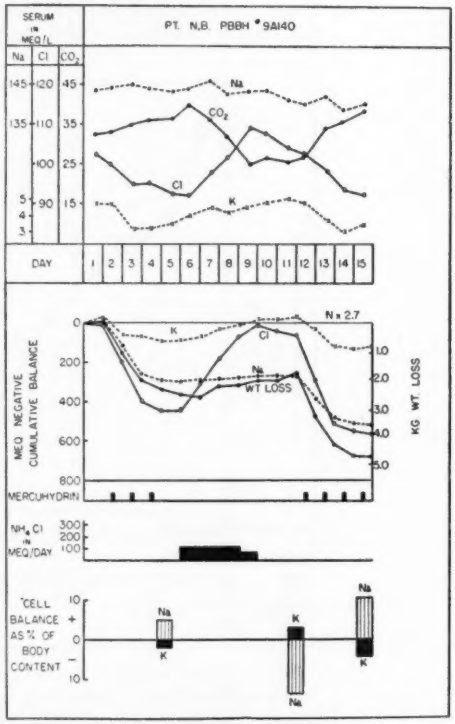

FIG. 3. SERUM ELECTROLYTE CONCENTRATIONS, EXTERNAL AND INTERNAL BALANCES IN RELATION TO THERAPY FOR PATIENT N. B. (TABLE IV) SHOWING A PATTERN SIMILAR TO THAT FOR PATIENT F. C.

The data on a massively edematous patient (V. S.), who lost 33 kg. of weight during a 22-day period of observation, are presented in Table II. Figure 1 is a graphic presentation of part of these data. Serum analyses showed an elevation of serum bicarbonate concentration and a reduction in chloride concentration prior to diuresis. On the first day of diuresis an additional elevation of the bicarbonate concentration occurred. After this initial rise, however, patient V. S. continued to diurese without progression of the serum changes. These variations from normal concentrations were not seen in the other patient (B. C.), whose diuresis was of the same type as that described in Figure 1.

The diuretic was administered at intervals throughout the period of study. At each interruption of treatment with mercury, loss of electrolyte and of body weight quickly stopped. Resumption of treatment resulted in renewed diuresis. At no time was the patient unresponsive. The negative balance of sodium was always greater than that of chloride and the ratio of the losses was approximately the same as the ratio of sodium to chloride in extracellular fluid. The course of the cumulative nitrogen and potassium balance indicated that intracellular fluid remained undisturbed. The curves for weight loss and sodium loss nearly coincided, a further indication of the extracellular nature of the fluid loss. The slight deviation of the two curves, as well as the indicated shift of sodium into cells, could well be accounted for by unmeasured losses. The pH of the urine remained between 6.4 and 7.7 during the course of the diuresis. No significant changes occurred in the daily total excretion of titratable acid or ammonia.

The data on two patients (F. C., N. B.), who at first responded to the administration of the diuretic but quickly became unresponsive, are given in Tables III and IV and are graphically shown in Figures 2 and 3. In both of these patients the negative balance of chloride exceeded the negative balance of sodium. Hypochloremic alkalosis developed without change in serum sodium concentration. Unlike the subject (V. S.) previously described, a negative balance of potassium occurred in the initial phase of diuresis and the concentration of serum potassium decreased on the initial day. As serum chloride concentration fell and bicarbonate concentration rose both subjects became refractory to further administration of the diuretic.

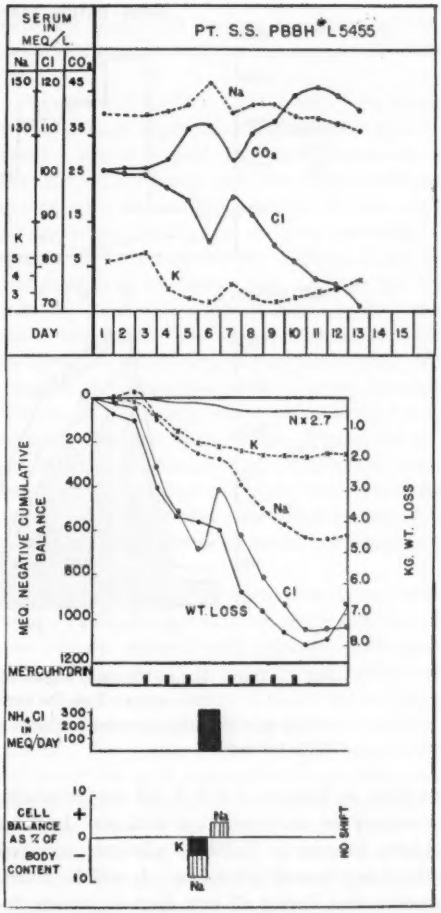

FIG. 4. SERUM ELECTROLYTE CONCENTRATIONS, EXTERNAL AND INTERNAL BALANCES IN RELATION TO THERAPY FOR PATIENT S. S. (TABLE V) SHOWING CONTINUED RESPONSIVENESS TO THERAPY DESPITE DEVELOPMENT OF HYPOCHLOREMIC ALKALOSIS

The administration of ammonium chloride or hydrochloric acid returned the serum concentrations of bicarbonate and chloride to normal. Both subjects once again responded to treatment with mercury but further diuresis resulted in repetition of essentially the same cycle.

The potassium loss noted in the two patients

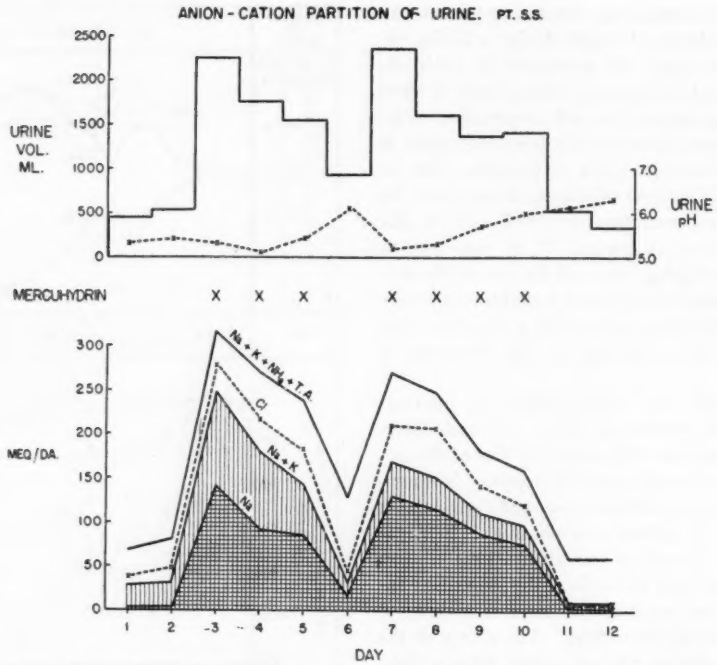

FIG. 5. DAILY URINE VOLUME, URINE pH AND THE ANION-CATION PARTITION OF THE URINE DURING TWO DIURETIC PERIODS FOR S. S. (TABLE V)

Note that chloride excretion is always greater than the sum of excretions of sodium and potassium.

described in Figures 2 and 3 did not invariably accompany the development of alkalosis. In three subjects (shown in Table I) alkalosis occurred without any loss of potassium. It will be noted, however, that during all four diuretic periods described for F. C. and N. B., potassium was lost in excess of nitrogen and in excess of the small amounts that would be expected from the simple loss of extracellular fluid. The physiologic significance of this loss is difficult to evaluate but may be assessed in terms of the percentage loss of estimated total body potassium. Patient N. B., at the end of the period of study, had lost approximately 6% of her estimated total body potassium. In her the potassium losses were repaired from a fixed intake during the period of chloride administration. In F. C., however, the potassium losses during each period of diuresis were additive and the net loss at the end of the experiment was more than 10% of the estimated total body potassium, yet there were no clinical or electrocardiographic evidences of potassium deficiency and the serum potassium level was within normal limits.

Examination of the relation of the actual weight loss to theoretical weight loss calculated from sodium showed that intracellular fluid as well as extracellular fluid was lost. During each period of diuresis in both subjects the weight loss calculated from sodium was seen to be less than the observed weight loss, the difference roughly approximating the amount that might be calculated from the loss of potassium associated with the loss of intracellular water. The difficulties that arise from attempts to predict shifts in water by calculation from observed body weights and balance measurements for electrolytes have been stressed by Yannet and Darrow (13) and Elkinton and coworkers (8). For this reason no attempt was

made to quantitatively assess the relationship of potassium loss to intracellular fluid loss. The inconsistencies that would be encountered are particularly evident in Figure 2, where it is seen that during the periods when hydrochloric acid was administered, increments in body weight occurred without simultaneous retention of either sodium or potassium. It may be postulated that changes of this nature are related to an "activation" of cellular cation, but they can probably be more reasonably explained as resulting from changes in dissociation of intracellular anion (phosphate) and a consequent increase in the number of osmotically active particles (14). The additional possibility that there is a primary disturbance in the excretion of water can not be ruled out. The degree of retention would be expected to produce only a slight change in tonicity, difficult to identify by measurements of serum sodium concentration.

The intracellular balances seen in Figures 2 and 3 show that during the development of alkalosis, sodium regularly tended to shift into the cells and potassium tended to leave them. In most instances the reverse occurred during administration of ammonium chloride or hydrochloric acid. These shifts are barely significant numerically, but the uniformity of their direction suggests that some such redistribution is taking place. The meaning of such shifts is not clear but they may serve the purpose of limiting the degree of relative acidosis or alkalosis in the extracellular fluid (3).

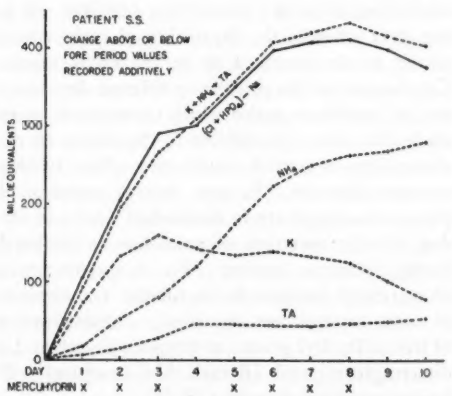

FIG. 6. CUMULATIVE URINARY EXCRETIONS ABOVE FOREPERIOD EXCRETIONS FOR PATIENT S. S. (TABLE V) PLOTTED TO INDICATE THE QUANTITIES OF POTASSIUM AND AMMONIUM COVERING THE SURPLUS OF CHLORIDE ABOVE SODIUM DURING THE SUCCESSIVE DAYS OF THE DIURESIS

Figure 4 and Table V (S. S.) depict the course of the balance study in a patient who also responded with a loss of chloride in excess of sodium but who despite progressive alkalosis did not become refractory. The changes in the serum concentrations are similar to those occurring in patients F. C. and N. B. (see Figures 2 and 3). Administration of ammonium chloride on the sixth day resulted in a partial return toward normal of the serum values, as well as remission in the symptoms of lethargy and anorexia which had appeared as diuresis proceeded. During the initial diuresis approximately 5% of the estimated body potassium was lost in the urine. During the second diuretic period no loss of potassium occurred that could not be accounted for in the extracellular fluid which had been lost. Alkalosis continued to increase without loss of potassium as diuresis proceeded.

Although the signs of congestive failure were rapidly diminishing as diuresis continued, the patient became anorexic and lethargic once again and gradually lapsed into coma during the final two days of observation. Several hours before death, intermittent ventricular tachycardia and shock developed. The patient died on the 13th hospital day with no evidence of congestive failure other than mild peripheral edema. Postmortem examination did not provide an explanation of the death. It seems possible that the fatal outcome may have been related to the severe imbalance in the composition of the body fluid. Although 5% of the estimated body potassium had been lost in excess of nitrogen and there was an abnormally low serum concentration of potassium, there were no distinct clinical evidences of potassium deficiency and the electrocardiogram did not show signs of hypokalemia. Another factor contributing to the terminal shock-like state in this patient may have been the rapid and severe depletion of the body fluid volume. In the end phases of observation water retention appeared with a progressive decrease in the concentration of serum sodium. Although clinical deterioration was observed in this patient and in one other patient with alkalosis (J. C.) several patients, not included in the present data, have been observed to have values for se-

rum pH of from 7.55 to 7.57 and total carbon dioxide concentrations of from 50 to 60 mEq./l. without the development of untoward symptoms and signs (15).

In Figure 5 a graphic analysis of the factors of anion-cation excretion in the urine of subject S. S. is presented. In the upper half of the chart the daily volumes and measurements of pH are shown. In the lower section the uppermost line records the daily fixed cation excretion plus urine ammonium and titratable acidity. Although the two relatively small components of fixed cation outgo in the urine, calcium and magnesium, are not included, sodium plus potassium plus ammonium plus titratable acid may be taken as approximately measuring the total cation excretion. By reference to the top line the anion excess over sodium plus potassium which is covered by ammonium plus titratable acid is defined. The relatively very small value for the unmeasured anions, phosphate, sulfate and organic acids, is shown by the proximity of the chloride measurements to this line.

In both periods of administration of Mercuhydrin urine volume is seen to increase greatly on the first day and then decline progressively, although a quite large diuretic effect continued to be obtained, and there was roughly corresponding change in the outgo of chloride and sodium in the urine. In the first period as may be seen by reference to the values before diuresis (days 1 and 2) removal of potassium was extensively increased whereas in the second period urine potassium was not above the initial value. This is explained by the larger values for ammonium and titratable acid over the second period.

The relation of potassium removal, ammonium production and urine acidity to the huge excess of chloride over sodium in the urine, which is the outstanding feature of the situation, is shown again in Figure 6. In this figure the daily values for change above or below the foreperiod measurements are recorded additively. The solid line defines, on this basis, anion excess composed of chloride plus phosphate over sodium. Measurements of sulfate and organic acids were not obtained. There was, as shown by the measurements in Table V, an increase of urine phosphate during the first mercuhydrin period along with the increased removal of potassium. The values for potassium, ammonium and titratable acid are recorded separately. Taken together, as shown by the uppermost broken line, they quite closely follow the anion excess over the sodium. The large removal of potassium above the foreperiod level during the first period of diuresis is quite clearly referable to the lag in ammonium production with the result that intracellular fixed cation is required to complete the coverage of anion excess over sodium. After the third day the increase of ammonium plus titratable acid permits the excretion of potassium to fall below the foreperiod value and there is gradual recovery of the potassium lost over the first three days. It should be noted that in some patients the discrepancy between chloride and sodium may be made up entirely by potassium without significant change in excretion of ammonium or titratable acid during the period of diuresis. This is illustrated particularly well in the data shown in Table IV (N. B.).

DISCUSSION

By means of direct observations on the kidney and short-term clearance studies investigators have identified many of the renal effects of mercurial compounds. The diuretic action of these drugs may be accounted for by a direct renal action (16, 17). Diuresis after single doses of mercurial compounds is not associated with an increase in the renal blood flow or in glomerular filtration rate but is apparently the result of a decreased tubular reabsorption of certain electrolytes (18–20). It is not clear whether the depression of reabsorption occurs in the proximal or in the distal tubule. Conclusions on this point have differed, depending on the conditions under which observations were made (21, 22). In addition to the effects on reabsorption, mercurial compounds affect tubular secretory function. In man, tubular secretion of para-amino-hippurate is diminished (23); in the dog, tubular secretion of potassium is inhibited during potassium loading (24). A specific action of mercurial compounds on tubular reabsorption of water has not been described. Administration of free sulfhydryl groups in compounds such as 2,3 dimercaptopropanol (British Anti-Lewisite) will inhibit mercurial diuresis (25–27).

The results in the present studies, as well as those of Blumgart and associates (1, 19) in normal subjects and patients with congestive heart failure may be interpreted to indicate that mercury

specifically depresses the tubular reabsorption of chloride and that cation excretion follows passively. The quantity of body fluid removed, however, is more closely related to fixed cation removal than to anion removal. In each period of diuresis the loss of sodium plus potassium is nearly equal to that predicted from the weight loss and concentration of these ions in the body fluids.

In a minority of the patients the explanation that sodium excretion is secondary to chloride excretion apparently does not account for the findings during diuresis. In these, sodium and chloride were lost in the proportion found in extracellular fluid. It should also be noted that, even in the patients who lost more chloride than sodium, the ratio of these losses varied widely. The factors responsible for the variation are not clear and require further study before a generally applicable concept describing the action of mercurial diuretics can be stated. It is possible that in every instance the effect of mercury is primarily on the reabsorption of chloride and that under optimal circumstances sodium will be lost with chloride in proportions appropriate to the maintenance of normal body fluid composition. However, if the stimuli that lead to sodium retention are intense because of hormonal factors or disturbances in renal hemodynamics, or both, then the factors that control normal cation-anion balance might become secondary to the demands of sodium retention, and chloride would be lost in excess of sodium. It has recently been suggested that changes in plasma volume may exert controlling effects on the excretion of salt and water (28). According to this view, a decrease in plasma volume leads to retention of sodium and water. In some of the present group of patients, the intensity of the sodium-retaining response may, in part, have resulted from a decrease in plasma volume consequent upon the initial diuretic response to the mercurial. Under these circumstances, the rate at which interstitial fluid is withdrawn into the vascular compartment would play an important role in determining the Na/Cl loss.

The tentative hypothesis that mercurial diuretics have their primary action on the tubular reabsorption of chloride is inferred from the observations presented here which indicate that, over the course of days, mercurials lead to a disproportionate removal of chloride from the body fluids. Other workers have suggested that the action of mercury may be primarily on proximal (22) or distal (21) tubular reabsorption of sodium. Such hypotheses find neither support nor refutation in the present data and their final evaluation must await further investigation.

Whatever the mechanism may ultimately prove to be, the data presented here indicate a close temporal relationship between the fall in serum chloride and rise in serum bicarbonate concentrations and the development of unresponsiveness to the mercurial. Repair of the chloride deficit and the concomitant alkalosis has regularly been accompanied by the return of responsiveness.

Although the evidence presented here suggests that in the unresponsiveness to mercurial diuretics the electrolyte disturbance is of critical importance, data of others suggest that the essential factor may be a reduction in glomerular filtration (21, 29). It is possible that reductions in glomerular filtration rate and serum chloride concentrations both produce unresponsiveness by diminishing the rate at which chloride is delivered to the tubules.

In the two patients whose losses during diuresis were of the same composition as extracellular fluid, responsiveness continued until edema had disappeared. One of them initially demonstrated hypochloremia and an elevated bicarbonate concentration of a degree often found in the refractory group. These abnormalities in this patient persisted throughout the period of study, suggesting that unresponsiveness may be more related to changes in concentration than to absolute levels. In this minority group of responsive patients no significant changes occurred in the rate of excretion of ammonium, potassium, or titratable acid.

In those patients who excreted chloride in excess of sodium during diuresis, the need for additional cation was met by the loss of potassium and ammonium in reciprocally variable quantities, and in some instances indirectly by an increase in the excretion of titratable acid. In many patients large amounts of titratable acid and ammonium continued to be excreted in the urine during the progression of the alkalosis. The production of an acid urine in the presence of alkalosis has previously been observed in conditions characterized by dehydration and sodium depletion and has been considered to represent urgent need for cation conservation by

the body (30, 31). During the production of experimental potassium deficit in animals metabolic alkalosis develops (32). One explanation of this event could be the loss of acid in the urine, as is suggested by the observations made during presumed potassium deficit in man by Kennedy, Winkley, and Dunning (33) and by Broch (34). Other mechanisms that might account for the production of alkalosis are (*a*) the exchange of hydrogen and potassium ions between extra- and intracellular fluid, or (*b*) a loss from the intracellular space of potassium and the anion of some weak acid. That one of the latter two mechanisms may be operative is suggested by the data describing the course of patient N. B. (Table IV). Elevation of serum pH and a progressive rise in serum bicarbonate accompanied by a fall in serum chloride concentration occurred without an appreciable increase in the excretion of ammonia or titratable acid. The urine remained close to neutrality as the chloride lost in excess of sodium was excreted with an almost equivalent amount of potassium. The observation that the intracellular pH of muscle tends to fall during imposed alkalosis likewise supports the possibility of internal transfers (35).

The loss of potassium finds its most ready explanation in the discrepancy between the excretion of sodium and chloride. In certain patients, as has been shown, potassium appears to act as a temporary source of cation until ammonium production is sufficiently increased to conserve fixed cation. In other patients potassium continues to furnish a large portion of the cation throughout the period of diuresis.

Two other possible mechanisms suggest themselves as factors in the potassium loss, though neither of these explains the data in all instances. Depression of the tubular reabsorption of potassium by mercurials has been shown to occur in the dog (24). If this occurs in man the effect must be transitory or irregular since 24-hour periods of observation in the present group of patients often indicated no loss of potassium. A third explanation, as suggested by the work of Darrow and associates (32), might be that the loss of potassium is the result of a biological equilibrium attained when normal renal function is carried out in the presence of deficit of certain ions in the body fluids. However, in the present group of patients, renal function cannot be considered normal and the deficits of potassium either did not occur or were of a much smaller degree than those demonstrated by these authors.

It should be pointed out that, even when there is a loss of potassium in excess of nitrogen, the deficits rarely appeared to be of clinical significance. Electrocardiograms were obtained on all patients during successive phases of the observations and were at no time suggestive of potassium deficit. Except for one instance, as long as the intake of food was adequate, the deficits of potassium were quickly repaired when mercurial administration was stopped. The possibility remains, however, that during a prolonged period of diuresis, a patient may become potassium deficient, particularly if his intake of food is low.

It has long been known that acidifying salts potentiate, and alkalinizing salts depress mercurial diuresis (2, 36, 37). The potentiation with acidifying salts has been found to be synergistic rather than additive. Recent studies in dogs by Axelrod and associates suggest that a change in pH is not the leading factor (38). Reduction in pH by the administration of ammonium chloride is followed by a synergistically potentiated response to mercurials but a comparable reduction in pH induced by breathing 7% carbon dioxide does not enhance the diuretic effect (38). It would appear from the present data that it is the anion pattern of the serum that is important in determining the diuretic response. That acidifying salts containing anions other than chloride potentiate diuresis may be explained by postulating that these anions either (*1*) substitute for chloride, or (*2*) allow for an increase in the concentration of chloride in the body fluids. Evidence for the second viewpoint is provided by the studies of Gamble and associates (39). These authors have shown that the administration of ammonium sulfate leads to elevation of the serum chloride concentration. Review of their data indicates the explanation of this phenomenon is that the diuresis induced by sulfate leads to an obligatory removal of cation and water without proportional loss of chloride.

It should be noted that in the present group of patients the reduction in serum chloride concentration was in no instance accompanied by a fall in serum sodium concentration while diuresis was occurring. The occurrence of hypotonicity after mercurial diuresis has, however, been reported by

many authors (40–42). The hypochloremic alkalosis seen in the present patients must be differentiated from the true "low salt" syndrome. Hypotonicity failed to develop in the present patients because water and cation were removed in proportional amounts. The development of hypotonicity requires the removal of cation without proportional quantities of water, the retention of water alone, or changes in the state of dissociation of certain intracellular compounds. Although hypertonic salt solution has been suggested for use in the management of hypotonicity associated with mercurial diuresis, there appeared to be no indication for its use in the group of patients here described.

SUMMARY AND CONCLUSIONS

The balance of weight, nitrogen, and electrolytes, the electrolyte changes in the serum, and the cation-anion pattern of the urine were studied in 10 patients with congestive heart failure who were undergoing mercurial diuresis.

The pattern of the balances was interpreted in terms of body composition and over-all function of the kidneys.

A minority of the patients lost water and electrolytes in a manner indicating that the only significant change in the body fluids was loss of extracellular fluid of usual composition. This small group of patients never developed mercurial unresponsiveness.

The majority of patients lost electrolytes and water in proportions that led to chloride depletion and alkalosis without change in the concentration of sodium in the serum. This type of response was often associated with a loss of intracellular fluid and potassium as well as with reduction of serum potassium concentration. In this group unresponsiveness to the diuretic developed in all but one instance. Repair of the chloride deficit with ammonium chloride or hydrochloric acid restored mercurial responsiveness. The serum concentrations of chloride and bicarbonate associated with unresponsiveness varied from patient to patient. In those patients in whom chloride excretion exceeded sodium excretion the surplus of chloride was covered by potassium and ammonium.

It has been suggested that the usual renal response to mercurial diuretics may result from interference with tubular reabsorption of chloride and that the loss of fixed cation is secondary to the effect on anion excretion. Possible factors determining the ratio of sodium to chloride loss have been discussed.

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POTENTIATION OF DIURETIC ACTION OF MERCUHYDRIN [1] BY AMMONIUM CHLORIDE [2]

By JAMES G. HILTON [3] with the technical assistance of HELEN KALINSKY

(From the Research Service, First Division, Goldwater Memorial Hospital, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York City)

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Potentiation of the diuretic action of mercurials by acidifying salts is well known (1–7). That the effect is synergistic and not merely additive was conclusively demonstrated by Ethridge, Myers and Fulton (8). The mechanism of this synergism is not well understood, and reports as to its nature are conflicting. Acidosis as the responsible factor has been emphasized by several investigators (1, 4, 8). This view also received support when it was noted that the administration of alkalinizing salts actually diminished the efficacy of mercurial diuretics (8). There are others, however, who observed potentiation of the mercurial effect persisting in the presence of a normal plasma acid-base balance in edematous patients receiving prolonged ammonium chloride administration (6, 7). Blumgart and associates reported a case in which the correction of a severe acidosis by alkali therapy restored responsiveness to a mercurial diuretic in a nephritic patient (9). Most recently, it has been noted that in dogs rendered acidotic by inhalation of 7% CO_2 there is no enhanced activity from the injection of a mercurial diuretic (10).

Since the one study of the combination of mercurials and ammonium chloride in normal individuals did not deal with effects on plasma acidity (11), it was considered of interest to determine whether any synergism would continue to exist after compensatory return of the plasma acid-base balance to normal. This normal balance can be achieved during prolonged administration of the salt to subjects in whom there is no disturbance in renal function or in fluid and electrolyte excretion. The mechanisms of this compensation have been investigated and thoroughly discussed elsewhere (12–14).

SUBJECTS AND MATERIALS

Twenty-four-hour urine volume and sodium, potassium, chloride and ammonia excretion were measured for a period of 80 consecutive days in five normal young adult male subjects. Fluid intake was allowed *ad lib.* but sodium chloride intake was held constant at 6 gms. per day. Arterialized venous blood samples (15) were drawn every seven days (three hours after the morning meal) and analyzed for pH, carbon dioxide content, sodium, potassium and chloride. Plasma bicarbonate was calculated from pH and carbon dioxide content by means of the Henderson-Hasselbach equation, using a pK of 6.1 for carbonic acid. Urine pH was also determined at the same time from a small specimen of urine voided directly into mineral oil. The chemical methods employed have been described in an earlier publication from this laboratory (16).

Each subject received an intramuscular injection of 2 ml. of Mercuhydrin at seven-day intervals throughout the 80-day period of study. The injections were given in the morning immediately following the withdrawal of the blood sample. After three control injections, ammonium chloride (uncoated tablets) was administered in a dosage of 2 gms. t.i.d. (with each meal) and continued for 30 days. The timing was such that mercurial injections were given on the days of maximum acidity and alkalinity of the plasma, respectively three days after starting and three days after stopping the ammonium chloride administration. A total of 11 mercurial injections were given to each subject during the 80-day period—three control, four during ammonium chloride administration, and four additional control injections.

RESULTS

The plasma and 24-hour urine electrolyte values for each day Mercuhydrin was given are tabulated in Tables I and II. It can be seen that the diuretic effect on water, sodium and chloride excretion is enhanced two-fold during the phase of ammonium chloride administration. Potassium excretion following mercurial injections during the two control periods (injection Nos. 1–3 and 8–11) did not differ from days on which no mercurial was given.

[1] Meralluride sodium.

[2] This study was supported in part by a grant from the Public Health Research Institute of the City of New York, Inc.

[3] Fellow of the New York Heart Association; present address, Dept. of Medicine, St. Luke's Hospital, New York, N. Y.

TABLE I

Potentiation of mercurial diuresis maintained in presence of normal plasma acid-base balance achieved during prolonged NH_4Cl administration

		CONTROL			NH_4Cl 113 mEq/day				CONTROL			
Mercurial Injection No.[a]		1	2	3	4	5	6	7	8	9	10	11
Subj. Bo.	pH[b]	7.40	7.41	7.40	7.33	7.35	7.38	7.40	7.43	7.39	7.39	7.40
	HCO_3^-[c]	27	28	25	19	27	28	27	29	26	27	27
	Na^+[c]	136	136	134	134	136	134	137	138	138	137	139
	Vol.[d]	1380	1680	1550	2400	2400	2790	2820	1230	1570	1370	1700
Subj. Cu.	pH	7.41	7.41	7.40	7.31	7.36	7.37	7.39	7.43	7.39	7.40	7.40
	HCO_3^-	29	27	26	21	25	27	26	30	27	26	26
	Na^+	140	136	135	138	137	137	140	142	141	130	141
	Vol.	1560	2600	1700	3600	3320	3360	3550	2250	1900	2600	2520
Subj. La.	pH	7.40	7.41	7.41	7.32	7.40	7.41	7.41	7.44	7.41	7.41	7.40
	HCO_3^-	24	25	24	17	24	24	23	29	25	25	26
	Na^+	140	137	137	136	135	135	137	138	137	136	137
	Vol.	2850	2970	2570	4080	3810	3650	3650	3000	2100	2480	2280
Subj. Ni.	pH	7.40	7.40	7.39	7.32	7.36	7.38	7.39	7.43	7.39	7.41	7.40
	HCO_3^-	27	24	24	20	24	26	26	30	26	25	25
	Na^+	142	138	138	137	137	137	138	138	141	140	141
	Vol.	1200	1380	1170	2100	3000	2100	2700	970	1200	1100	1140
Subj. Wr.	pH	7.41	7.41	7.40	7.33	7.35	7.37	7.40	7.44	7.40	7.40	7.39
	HCO_3^-	27	25	27	19	23	26	26	30	26	26	26
	Na^+	140	137	138	137	137	136	139	139	140	139	139
	Vol.	1160	1180	1350	2850	3040	3220	3310	1420	1300	1480	1100

[a] 7 days interval between injections. Nos. 4 and 8 respectively 3 days after starting and 3 days after stopping NH_4Cl.
[b] Plasma pH.
[c] Plasma values in mEq/L.
[d] Urine volume in ml/24 hours.

TABLE II

Effect of mercurial diuretic on electrolyte excretion during prolonged NH_4Cl administration*

		CONTROL			NH_4Cl 113 mEq/day				CONTROL			
Mercurial Injection No.		1	2	3	4	5	6	7	8	9	10	11
Subj. Bo.	Na^+	206	184	189	266	252	258	302	180	172	184	192
	NH_4^+	24	40	20	85	87	79	80	33	36	35	40
	Cl^-	202	184	199	365	358	366	413	170	211	197	208
	K^+	42	48	53	66	76	76	84	53	59	46	55
Subj. Cu.	Na^+	144	190	130	302	268	252	334	125	102	153	140
	NH_4^+	23	35	30	110	98	96	94	37	30	42	45
	Cl^-	172	188	143	440	400	370	460	130	118	184	141
	K^+	42	51	53	73	73	66	82	48	50	48	49
Subj. La.	Na^+	300	302	270	430	395	350	364	359	228	277	267
	NH_4^+	16	18	22	90	90	92	99	29	37	19	21
	Cl^-	328	319	309	540	500	450	491	387	236	281	294
	K^+	95	99	95	119	95	95	109	61	96	88	111
Subj. Ni.	Na^+	162	152	182	212	289	208	320	132	149	121	166
	NH_4^+	26	37	24	82	85	87	86	29	24	30	32
	Cl^-	171	175	177	328	449	325	435	150	162	145	172
	K^+	79	54	57	82	116	92	97	60	84	64	56
Subj. Wr.	Na^+	228	212	227	413	354	377	440	260	262	252	241
	NH_4^+	24	28	29	83	85	104	98	26	29	32	33
	Cl^-	241	222	249	519	500	540	554	264	252	276	245
	K^+	39	45	49	70	82	86	91	35	62	50	40

*All values in mEq/24 hours. This table is constructed identically to Table 1.

The slight increase during the period ammonium chloride was given (injection Nos. 4–7) was compensated for by decreased excretion on the day following diuresis, and there was no change detected in any of the weekly plasma levels.

It is apparent from the data shown in Tables I and II that the potentiating effect persists despite return of the plasma acid-base balance to normal. The degree of potentiation observed when there was no acidosis present (injection No. 7) was equal to that during the most acidotic period (injection No. 4). Plasma chloride, not tabulated, in every instance reciprocated the changes in plasma bicarbonate. There was no significant change in plasma sodium at any time during the period of study. Retention of sodium and chloride occurred for two to three days following each diuretic day, and was most marked during the period of potentiated injections. This is shown in Figures 1 and 2 in which complete data on water, sodium and ammonia excretion from two representative subjects have been charted. It can also be seen that ammonia excretion was unaltered by the mercurial injections.

Urinary pH in all subjects was between 4.5 and 5.0 during the administration of ammonium chloride. In four instances, this represented a drop of about one pH unit from control values. In Subject Ni. urine pH, low to begin with, did not drop further upon administration of the salt. Urinary titratable acidity was not determined; but it is possible that it was increased during this period since, in terms of the chloride ingested as ammonium chloride, ammonia synthesis by the kidney was

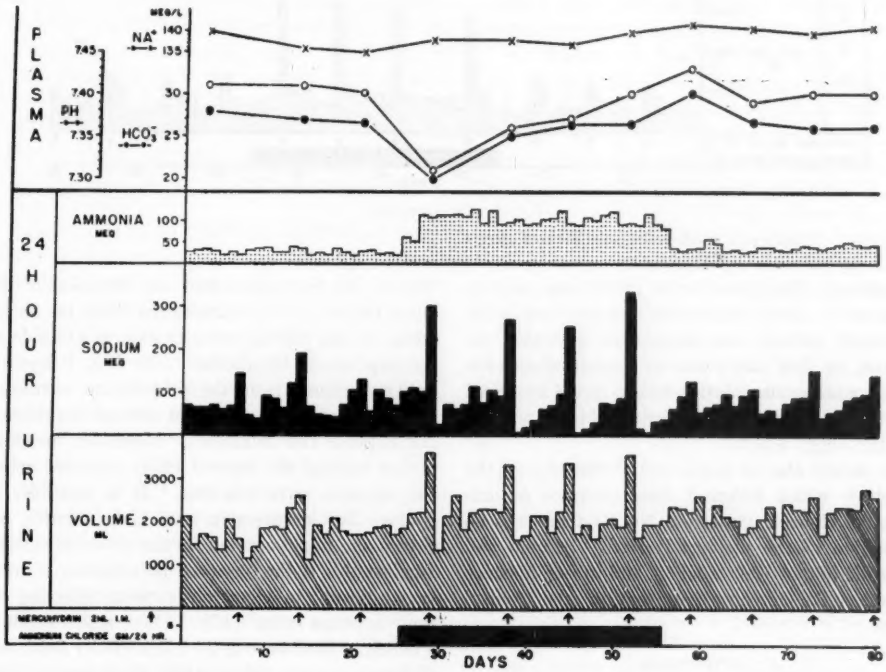

FIG. 1. SUBJECT CU.

Twenty-four-hour excretion of ammonia, sodium, and water is plotted in the lower part of the figure; plasma pH, sodium, and bicarbonate concentrations in the upper part. Note enhanced effect of Mercuhydrin injections (indicated by the arrows) on sodium and water excretion during the entire period of ammonium chloride administration, irrespective of changes in plasma pH and bicarbonate. Decreased excretion of sodium on the days following mercurial diuresis is also evident.

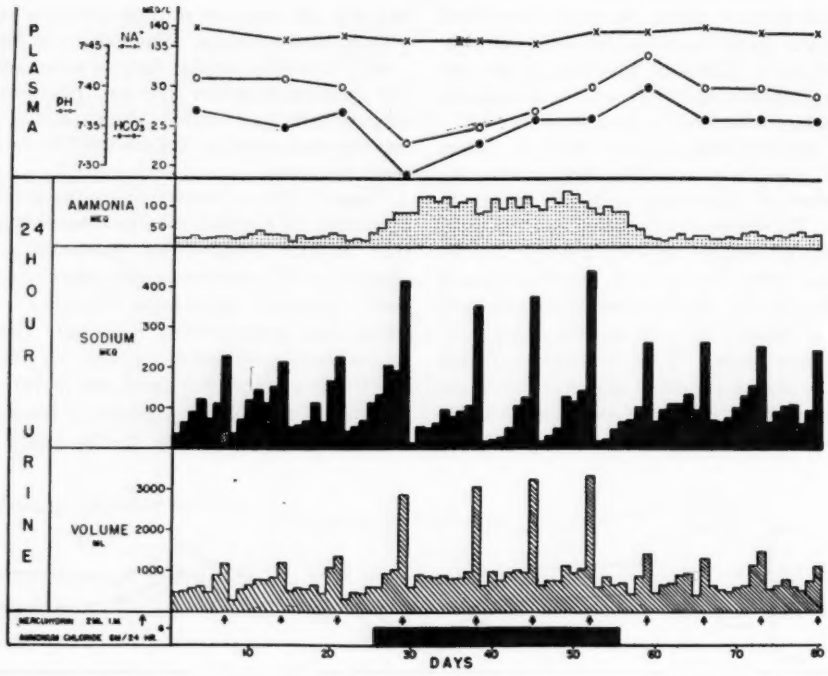

FIG. 2. SUBJECT WR.

This figure is otherwise identical to Figure 1.

consistently less than intake in all five subjects (Table II). It is also possible that increases in unmeasured calcium and magnesium excretion occurred, or that there was incomplete absorption of the ammonium chloride tablets in the intestinal tract. Either of these situations could well account for this slight deficit.

It should also be noted that at the time of the alkalosis which followed discontinuance of ammonium chloride (injection No. 8) there was no inhibition of the mercurial effect. Sodium and chloride output was equal to, and in one instance (Subject La.) greater than, that on other control days.

DISCUSSION

It is apparent that the synergistic effect of ammonium chloride on the diuretic action of Mercuhydrin is independent of the pH and bicarbonate concentration of the plasma. The data support the view (10) that acidosis *per se* is not the decisive factor. By the same token, an alteration of the anion pattern of the extracellular fluid, *i.e.* an increase in the plasma concentration of chloride at the expense of bicarbonate (10, 17), is equally unlikely inasmuch as the potentiation continued in the presence of subsequent normal bicarbonate and chloride concentrations. Moreover, no diminution beyond the control effect occurred when the subjects were alkalotic. It is possible, of course, that increases in total body chloride occurred and might account for the observed results. Against this is the observed potentiation of mercurial diuresis in edematous patients receiving cation exchange resins (18). These resins produce a deficit of fixed base in the extracellular fluid, and thereby cause an acidosis which when compensated is characterized by either normal or increased plasma chloride concentrations (18, 19). In any event, however, it is extremely unlikely that increases in total body chloride content occur under these conditions.

Furthermore, it has been shown that the administration of potassium chloride does not enhance the diuretic effect of certain organic mercurials (8). This has been confirmed by administering potassium chloride in amounts of 113 meq. per day to subject Wr. under conditions identical to those described earlier for the study of the effect of ammonium chloride. Ingestion of the potassium salt was begun four days following mercurial injection No. 11 (Table II) and continued for three weeks. The average 24-hour excretion rates of water, sodium and chloride for the control injections (Nos. 8–11) were 1,325 ml., 253 meq., and 259 meq., respectively (calculated from Tables I and II). The average excretion rates for these same substances during potassium chloride administration (three mercurial injections at weekly intervals) were 1,330 ml. water, 218 meq. sodium, and 341 meq. chloride. Plasma electrolyte pattern and acid-base concentration during this latter period did not differ from that of the control period. The increase in chloride excretion was covered by an equal amount of potassium. These observations demonstrate the absence of any enhancing effect of potassium chloride on the excretion of water and sodium following mercurial injections in the normal human subject.

The potentiating effect on sodium excretion cannot be accounted for on the basis of mercurial inhibition of ammonia synthesis since the 24-hour output on the days of mercurial administration did not differ from that on non-mercurial days. Moreover, during the two control periods, endogenous ammonia excretion was similarly unaltered.

Increased acidity of the urine with resulting increase in the concentration of ionized mercury within the tubular cell is a possibility that must be considered. It has been postulated that organic mercurials act by liberating mercury ions (20). The general acceptance of this concept has led to the view that the potentiating effect of acidifying salts is due to an increase in the dissociation of mercury from its organic complex although there is no real evidence to support such an idea. Actually, *in vitro* studies indicate that at least 40% of some organic mercurials would have to be ionized in order to account for their effects on this basis (21). Moreover, in experiments on the isolated rabbit kidney cortex, it has been shown that altering the pH of the medium has no effect on the degree of inhibitory action of Mercuhydrin on certain transport mechanisms (22). In order to implicate urine acidity as affecting the degree of dissociation of organic mercury, the assumption must be made that the mercury ion enters the tubular cell by reabsorption from the glomerular filtrate, and not directly from the blood stream in the course of secretion. If the latter were the case, persistence of the potentiating effect in the presence of normal plasma acid-base concentrations would be difficult to explain on the basis of an increase in the dissociation of the organic mercurial. In view of these considerations, and from the evidence that in one subject there was no change in urinary pH during the period of potentiation, there is little support for the concept that a change in the ionization of Mercuhydrin could account for the observed enhancement.

Small rises in filtration rate, undetectable by present clearance techniques, could conceivably be of significance. It has been shown that raising (23), or lowering (24), glomerular filtration rate profoundly alters the renal response to mercurial diuretics. A corollary to this, however, is that tubular function as regards absolute sodium and chloride reabsorption remains constant. This would seem to be a very unlikely occurrence.

The most reasonable hypothesis would therefore appear to be that some as yet undefined alteration in the metabolism of the tubular cells is an influencing factor. Whether this leads to the accumulation of a greater concentration of Mercuhydrin within the cell, or whether in some manner the equilibrium reaction between the mercurial and the susceptible enzyme is shifted towards the formation of a more stable complex is, of course, purely speculative.

SUMMARY

Data have been presented showing that in normal subjects potentiation of a mercurial diuretic by ammonium chloride administration is not dependent on any alteration in plasma acid-base balance.

The possible nature of this mechanism has been discussed.

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EFFECTS OF INJECTION OF HYPERTONIC GLUCOSE ON METABOLISM OF WATER AND ELECTROLYTES IN PATIENTS WITH EDEMA[1]

By ROBERT TARAIL,[2,3] DONALD W. SELDIN,[4] AND ALLAN V. N. GOODYER[5]

(From the Department of Internal Medicine, Yale University School of Medicine, and the Medical Service of the New Haven Hospital, New Haven, Conn.)

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In normal human subjects, rapid injection of hypertonic glucose produces severe hyperglycemia which elevates the effective osmotic pressure of the extracellular fluid, thereby withdrawing water from cells, expanding extracellular volume, and decreasing the concentration of serum sodium (1). Large amounts of salt as well as water are excreted in the urine as intense glycosuria supervenes (1, 2). Similar effects have been demonstrated with a variety of nonelectrolytes, predominantly sugars of small molecular size (1–6). The reasons for these accelerated urinary losses of salt and water are not clear. They have usually been ascribed to the osmotic influence within the renal tubules of that fraction of the injected solute which appears in the urine (2–6).

The following study of the effects of injections of hypertonic glucose in edematous cardiacs and cirrhotics was undertaken for two reasons: *1*) To determine whether this procedure might have therapeutic value in facilitating the delivery of edema or ascites ; *2*) To determine by comparison with normal subjects whether a different physiological background, which includes a great propensity toward renal retention of salt (*7*), conditions the response of the body fluids and kidney to osmotic loads.

METHODS AND CALCULATIONS

Chemical methods were identical with those previously reported (1, 3, 8), except that serum glucose was usually determined directly rather than estimated from the concentration of glucose in whole blood.

Balance data were calculated using methods and assumptions previously described from this department (1, 8). Changes in extracellular volume were estimated from alterations in the chloride balance after assuming an initial extracellular volume of 35% of the body weight. Balance periods, designated in Table II, represent intervals between withdrawals of specimens of blood. These periods, therefore, may include several collections of urine which are listed individually in Table I.

EXPERIMENTAL PROCEDURE

The subjects of the experiments were seven patients with severe edema, which in four (A. B., T. L., J. R., M. S.) was primarily related to cirrhosis of the liver and in three (J. W., R. J., L.) was cardiac in origin (Table I). One patient (L., Table I) had well-controlled diabetes. All patients had been maintained on a low-salt diet containing less than 1 gm. of salt per day for at least five days prior to the study. Those patients with congestive heart failure had been digitalized, and other diuretic measures such as bed rest and mercurials had been successful in only two patients of the seven. J. W. was given a mercurial 48 hours before the injection of glucose, with an excellent response; L. had been losing weight slowly as a result of bed rest, a salt-free diet, and readjustment of the dosage of digitalis.

The experiments were performed while the patients were thirsting (dehydration) or taking water (hydration). In the three dehydration experiments, food and fluid were withheld for 12 to 16 hours, after which control urines and bloods were obtained and a rapid infusion of hypertonic glucose was begun. The volume and concentration of the glucose solutions, as well as their rate of administration, are listed separately for each patient in Table I. Specimens of blood were then obtained at the time of voidings, shortly following cessation of the infusion, and on one or two occasions thereafter. Subjects were recumbent throughout the experiment. The same general procedure was followed in the four hydration experiments, except that the subjects ingested 150 to 300 cc. of tap water per hour for eight hours prior to and during the injection of glucose. R. J., however, was unable to drink water after the start of the infusion because of nausea. Mannitol clearance (presumably a measure of glomerular filtration rate) and para-aminohippurate clear-

[1] Read by title at the 42nd annual meeting of the American Society for Clinical Investigation, May, 1950.

[2] This work was done during the tenure of a Life Insurance Medical Research Fellowship.

[3] Present address: Department of Research Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

[4] Present address: Department of Medicine, Southwestern Medical College of the University of Texas, Dallas, Texas.

[5] Markle Scholar in Medical Science.

TABLE I

Effect of infusions of glucose on concentrations of electrolytes and glucose in serum, and on renal excretion of water, electrolytes, and glucose

Experiment	Hydration	Solution*	Period†	Time	Serum						Blood		Urine							
					Cl	Na	K	P	Gluc.	Osm.‡ conc.	Hb.	Hct.	Flow	Cl	Na	K	P	Gluc.	Urea§	Total** solutes
				min.	*mEq./L*	*mEq./L*	*mEq./L*	*mg./L*	*mM/L*	*mM/L*	*gm.%*	%	*cc./hr.*	*mEq./hr.*	*mEq./hr.*	*mEq./hr.*	*mg./hr.*	*mM/hr.*	*mM/hr.*	*mM/hr.*
1. A. B.††	Hydro-	1000 cc.	"C"	178	98.1	130	4.8	39.1	7	138	12.7	36.3	8	0.03	0.02	0.9	3	0	—	—
59.1 kg.	penia	25% Gluc.	1	49	89.5	110	3.7	29.0	37	133	9.7	34.0	282	0.6	0.1	4.2	32	62	19	89
Cirrh.		48 min.	2	25	—	—	—	—	—	—	—	—	654	5.1	0.5	6.8	39	153	21	189
			3	31	—	—	—	—	—	—	—	—	792	8.4	1.0	10.5	67	205	35	263
			4	44	94.3	127	2.6	41.8	6	132	11.2	32.8	234	1.4	0.06	2.6	43	72	20	97
2. T. L.††	Hydro-	1175 cc.	"C"	665	91.5	129	5.5	39.4	7	138	15.8	41.8	38	2.1	0.07	4.2	38	0	12	20
71.3 kg.	penia	20.8%	1	56	87.1	114	5.1	33.9	48	143	13.9	44.1	450	5.9	0.8	6.9	60	83	17	115
Cirrh.		Gluc.	2	27	—	—	—	—	—	—	—	—	722	16.5	2.4	13.7	66	162	25	219
		52 min.	3	86	91.0	132	4.3	34.1	13	142	15.8	50.0	161	3.7	0.1	3.6	48	41	13	61
3. J. R.	Water	800 cc.	"C"	82	97.8	132	4.0	43.8	5	139	15.4	48.4	189	1.1	0.2	1.6	19	0	9	12
91.0 kg.	Diur.	25% Gluc.	1	69	96.8	125	3.9	37.8	34	146	15.7	46.5	422	3.2	1.0	2.8	55	45	14	67
Cirrh.		39 min.	2	46	—	—	—	—	—	—	—	—	444	4.9	2.0	2.5	69	76	21	106
			3	58	98.6	130	3.2	39.7	10	138	16.9	52.3	132	0.7	0.2	1.5	56	16	9	28
4. M. S.	Water	800 cc.	"C"	112	99.0	132	5.4	49.4	6	140	16.0	46.3	79	0.5	0.6	1.4	24	0	9	13
68.7 kg.	Diur.	25% Gluc.	1	45	95.1	123	4.0	36.0	42	148	13.6	37.8	267	2.5	1.6	1.8	27	48	7	62
Cirrh.		36 min.	2	15	—	—	—	—	—	—	—	—	1232	16.6	8.2	10.0	118	218	38	292
			3	57	—	—	—	—	—	—	—	—	205	2.0	0.3	2.5	52	60	13	79
			4	86	100.0	132	4.1	39.7	4	138	16.1	47.5	31	0.5	0.1	1.3	35	3	6	12
5. J. W.	Hydro-	900 cc.	"C"	715	102.2	140	4.2	36.9	5	146	15.8	45.0	35	4.4	4.5	0.5	13	0	8	18
82.7 kg.	penia	25% Gluc.	1	93	97.6	129	4.3	33.9	39	153	14.8	44.8	181	16.6	17.4	0.7	32	22	15	73
Card.		90 min.	2	54	100.9	135	4.0	32.7	15	147	14.9	44.3	267	20.8	21.0	1.2	55	43	24	111
6. R. J.	Water	600 cc.	"C"	83	78.5	124	3.4	23.8	5	130	14.8	48.5	42	0.3	0.2	2.0	9	0	17	21
75.4 kg.	Diur.	25% Gluc.	1	40	76.9	112	3.1	23.3	28	129	13.0	44.6	338	1.6	1.3	3.8	39	64	37	111
Card.		30 min.	2	93	79.5	117	2.8	19.7	13	126	14.3	45.4	169	0.7	0.3	2.5	34	33	26	65
7. L.	Water	800 cc.	"C"	51	93.0	136	4.3	38.4	11	146	15.6	51.3	74	0.5	0.9	1.6	1	0	11	16
61.1 kg.	Diur.	25% Gluc.	1	45	87.0	128	4.3	41.1	58	161	14.8	50.6	620	22.4	26.4	5.4	3	81	24	169
Card.		43 min.	2	19	—	—	—	—	—	—	—	—	767	34.4	36.8	5.6	12	143	21	250
Diab.			3	39	—	—	—	—	—	—	—	—	343	7.2	8.9	2.7	9	83	15	121
mell.			4	37	91.8	130	4.2	37.2	34	151	15.0	50.9	227	1.2	1.6	1.8	6	63	14	83

* Injection started immediately after end of control period.
† Blood specimens drawn at end of corresponding urine-collection periods.
‡ Represents sum of serum concentrations of sodium and potassium plus ½ serum concentration of glucose.
§ Estimated from excretion of non-protein nitrogen by assuming urea nitrogen comprised 90% of this value.
** Represents sum of excretion rates of urea and glucose, and twice the sum of the excretion rates of sodium and potassium.
†† Clearances of mannitol and para-aminohippurate were measured shortly before the study in A. B. and T. L. Expressed as cc./min./1.73 m², mannitol clearances were 124 in A. B. and 94 in T. L.; PAH clearances were 647 and 526 respectively.

ance (presumably a measure of renal plasma flow) were measured in two subjects (A. B. and T. L.) shortly before this study.

Painful muscular cramps of the extremities occurred within one hour following the infusion in the three hydropenic subjects. These spasms were accompanied by paresthesiae and, in one instance, by painful hyperperistalsis. These phenomena persisted for several hours but did not occur in hydrated subjects. It is noteworthy that the rapid intravenous injection of hypertonic solutions into decompensated cardiacs did not produce any respiratory distress, although mild transient dyspnea was noted at the end of the infusion given to J. R. who had cirrhosis.

RESULTS

Analytical and derived data are presented in Tables I and II and in Figures 1–3.

Serum and urine during the control period (*Table I*). The concentration of serum sodium was low initially in five patients (A. B., T. L., J. R., M. S., R. J.), ranging from 123.7 to 132.2 mEq./L.[6] The serum chloride was within the normal range in all but one patient (T. L.). The serum potassium was normal in all patients. The serum phosphorus was low in all patients, ranging from 0.7 to 2.1 mg. per 100 cc. The serum glucose was normal in all patients.

[6] The low concentration of serum sodium was presumably a consequence of renal retention of water in excess of salt. This reduction was not the result of urinary losses of salt, since only negligible amounts were excreted during the control period as well as during other, unrecorded, periods of study. Extrarenal losses of salt could not be implicated, since there was no vomiting, diarrhea, or undue sweating.

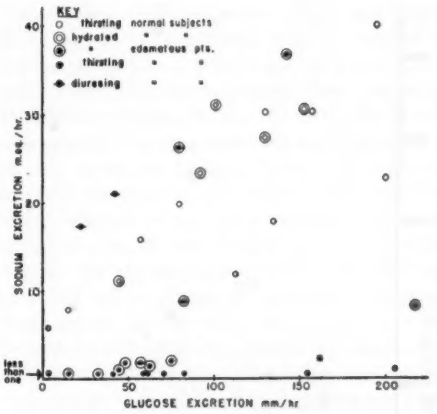

FIG. 1. THE RELATIONSHIP BETWEEN GLUCOSE EXCRETION AND SODIUM EXCRETION DURING GLUCOSE DIURESIS IN VARIOUS SUBJECTS

Note that the rates of sodium excretion were corrected for the initial rates prior to the administration of glucose. (Note also that a semi-solid circle with a horizontal bar indicates a value obtained in a diuresing, hydrated, edematous patient [L.].)

TABLE II

Balances of electrolytes and glucose; changes in distribution of body water

Experiment, Subject, Wt.	Balance period*	External balance†					Change in volume			Extracellular balance				Intracellular balance			
		Cl	Na	K	P	Gluc.	Extracell.‡	Intracell.§	Plasma**	Na	K	P	Gluc.	Na	K	P	Gluc.††
		mEq.	*mEq.*	*mEq.*	*mg.*	*gm.*	*liters*	*liters*	*%*	*mEq.*	*mEq.*	*mg.*	*gm.*	*mEq.*	*mEq.*	*mg.*	*gm.*
1. A. B.	A	− 3	− 3	− 4	− 26	+241	+1.9	−1.2	+36	−195	−16	−150	+123	+192	+12	+124	+118
59.1 kg.	B	−10	− 4	−10	− 68	− 40	−1.2	+0.3	−12	+205	−28	+237	−128	−209	+18	−319	+88
2. T. L.	A	− 9	− 5	− 6	− 56	+231	+1.4	−0.7	+ 9	−230	− 3	− 90	+196	+225	− 3	+ 34	+ 35
71.3 kg.	B	−19	− 9	−11	− 98	− 24	−1.6	+1.0	−23	+280	−28	− 49	−168	−289	+17	− 49	+144
3. J. R.	A	− 7	− 4	− 3	− 64	+191	+0.2	+0.1	+ 2	−250	− 2	−185	+164	+246	− 1	+121	+ 27
91.0 kg.	B	− 7	− 5	− 3	−107	− 13	−0.6	+0.7	−17	+130	−25	+ 38	−141	−135	+22	−145	+128
4. M. S.	A	− 5	− 4	− 1	− 20	+194	+0.9	−0.4	+36	− 90	−30	−292	+165	+ 86	+29	+272	− 29
68.7 kg.	B	−10	− 5	− 6	−129	− 21	−1.3	+1.4	−29	+150	− 3	+ 40	−173	−155	− 3	−169	+152
5. J. W.	A	−29	−31	− 1	− 50	+219	+1.1	−0.5	+ 7	− 98	+ 8	− 5	+178	+ 67	− 9	− 45	+ 41
82.7 kg.	B	−22	−23	− 1	− 49	− 7	−1.1	+0.8	0	+ 51	−13	− 57	−128	− 74	+12	+ 8	+121
6. R. J.	A	− 5	− 7	− 3	− 26	+142	+1.1	−0.8	+20	−130	− 5	+ 96	+115	+123	+ 2	−122	+ 27
75.4 kg.	B	− 3	− 3	− 4	− 52	− 9	−1.5	+1.2	−10	− 90	− 8	−212	− 80	+ 87	+ 4	+160	+ 71
7. L.	A	−18	−22	− 4	− 2	+189	+1.3	−1.0	+ 7	− 10	+ 6	+110	+195	− 12	−10	−112	− 6
61.1 kg.	B	−18	−22	− 5	− 13	− 25	−1.4	+1.5	− 2	−135	− 8	−140	−104	+113	+ 3	+127	+ 79

* Balance data are expressed per individual balance period rather than cumulatively. Duration of balance period is the interval between withdrawal of blood specimens, as shown in Table I.

† Balance of chloride and sodium are corrected for small quantities lost in serum drawn for analysis.

‡ Calculated from changes in chloride balance after assuming initial extracellular volume of 35% of body weight.

§ Estimated from measured oral and intravenous intake and urinary excretion of water, the calculated changes in extracellular volume, and assumed insensible water loss at the rate of 40 cc./hr. Since water of oxidation comprised less than 100 cc. per balance period, it was not included.

** Estimated from changes in hematocrit and in hemoglobin concentration.

†† Positive intracellular balances of glucose represent utilization or storage.

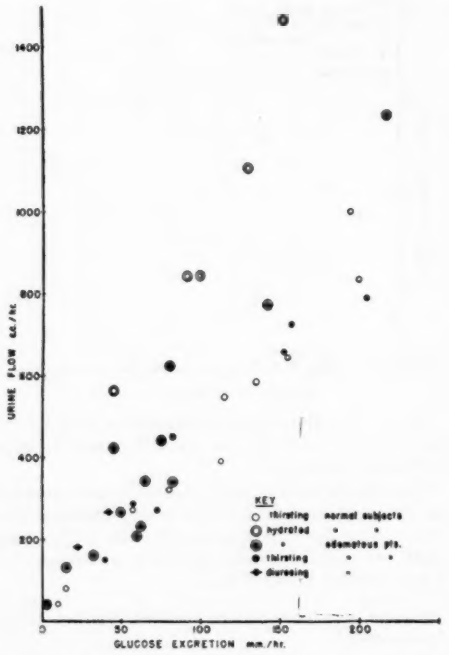

FIG. 2. THE RELATIONSHIP BETWEEN GLUCOSE EXCRETION AND URINE FLOW DURING GLUCOSE DIURESIS IN VARIOUS SUBJECTS

See legend of Figure 1 for significance of semi-solid circle with horizontal bar.

mal range or slightly low in three of these patients (A. B., J. R., M. S.), reduced in proportion to sodium in one (T. L.), and disproportionately reduced in R. J., a severely decompensated cardiac with primary respiratory CO_2 retention. The serum sodium was normal only in the patients who had responded to diuretic measures before the study period (see Procedure). Initial concentrations of serum potassium and inorganic phosphorus were slightly depressed in R. J. probably because of impairment of appetite and low intake of potassium and phosphorus.

The urine contained negligible amounts of sodium and chloride, except in the case of J. W. who was still undergoing a mercurial diuresis.

Body fluids and the concentration of serum sodium. Extracellular volume (chloride space) was expanded from 0.2 liter to 1.9 liters (in five of the seven patients the expansion exceeded 1 liter) shortly after cessation of the glucose infusion (Balance period A, Table II). The magnitude of this expansion was greater than could be accounted for by retention of injected or ingested fluid. In the three thirsting patients (A. B., T. L., J. W.), the increase in extracellular volume seemed to exceed the total amount of injected water, even if the water intake is not corrected for the greatly accelerated urine flow. Therefore, the expanded extracellular volume after the injections of glucose was dependent upon a transfer of water from cells into the extracellular fluid.

The concentration of serum sodium fell by 7 to 20 mEq./L at the end of the infusion of glucose. This fall was not the result of augmented excretion of sodium in the urine, since the concentration of sodium in the urine was far below that of serum. Although a transfer of sodium into cells (Table II) could account for part of the depression of the serum sodium, it should be emphasized that the error inherent in estimations of cellular transfers of sodium on the basis of alterations in the chloride space may be large (9). This is true not only because of the fact that sodium is

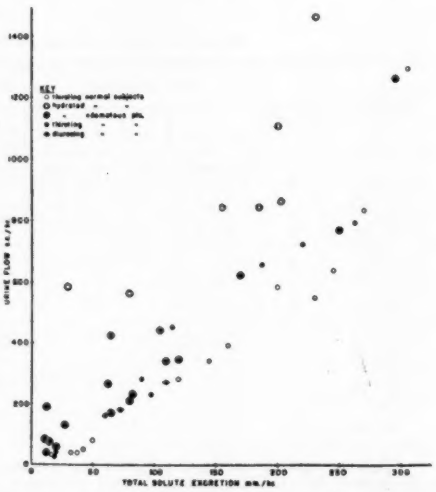

FIG. 3. THE RELATIONSHIP BETWEEN THE EXCRETION OF TOTAL SOLUTES AND URINE FLOW DURING GLUCOSE DIURESIS IN VARIOUS SUBJECTS

See legend of Figure 1 for significance of semi-solid circle with horizontal bar.

present principally in the extracellular fluid in high concentration, but also because of the probability that the chloride space is not precisely equivalent to extracellular volume (10, 11). Hence, comparatively slight errors in the measurement of changes in the extracellular volume, as well as in the chemical analyses, could result in large calculated transfers. This possibility of magnifying small errors by multiplication with large numbers is especially likely in the present study, since chloride space was assumed to be almost twice normal. Therefore, the error of the estimated movement of sodium into or out of cells would, other things being equal, be almost twice as great as that in a normal subject. It is probable, therefore, that the fall in the concentration of serum sodium was principally a result of dilution of the extracellular fluid by cell water (Table II) withdrawn from the intracellular phase by the marked increase in the effective osmotic pressure of the extracellular fluid produced by hyperglycemia.

As hyperglycemia receded, the effective osmotic pressure of extracellular fluid fell sharply, and the concentration of serum sodium rose to values approaching those of the control period. This rise in sodium concentration was probably a sequel of the return of water to the intracellular phase (Table II) in response to the new osmotic gradient.

Occasional discrepancies between estimated changes in effective osmotic pressure (Table I) and changes in intracellular volume (Table II) were noted. Although it may be tempting to invoke possible alterations in osmotic activity of components of the cellular phase to explain away these disparities, it is likely that these apparent changes in intracellular activity reflect cumulative chance errors in chemical methods and in the assumptions inherent in the methods of calculation.

Plasma volume, estimated from changes in hematocrit and hemoglobin values (Tables I and II) expanded during the infusion and contracted subsequently. Extreme changes in plasma volume seem to have occurred during certain periods, but these probably illustrate the crude nature of this measure of change in plasma volume. It is also possible that these apparent changes of plasma volume represent in part, temporary shifts of interstitial fluid into and from the vascular compartment presumably as a result of transient diffusion gradients of glucose.

Excretion of salt and water. Rapid injection of 600–1200 cc. of hypertonic glucose resulted in massive glycosuria (up to 39 gm. or 218 mM/hr.) and marked acceleration of urine flow (Table I). In only one subject (A. B.), however, did the volume of urine excreted following administration of glucose exceed the intake of water. If extrarenal losses of water were of the order of 40 cc./hr., the water balances of the other patients either approached zero or were frankly positive. Despite the fact that the excretion of sodium increased appreciably when glycosuria occurred, significant quantities of the ion were excreted by only two patients (J. W. and L.), mentioned above, who were already delivering their edema.

Transfers and excretion of potassium and phosphorus. The concentration of serum potassium fell in all patients except L. and J. W. from 0.1 to 1.4 mEq./L at the completion of the glucose infusion, and remained depressed (M. S.) or fell further (all other cases) in the subsequent periods (Table I). Balance data (Table II) indicate that in a majority of periods this fall was attributable primarily to a rate of liberation of potassium from cells which was diminished when compared with the control period, or to a net movement of potassium into cells, in conjunction with the utilization of glucose. In these patients, as in normal subjects (1), this movement of potassium toward cells counteracted the usual tendency for cells to discharge potassium to preserve a constant serum concentration in the face of increased urinary excretion and expanding extracellular volume. The excretion of potassium was augmented by the glucose infusion in all patients.

Changes in the concentration of serum inorganic phosphate usually paralleled those of serum potassium, particularly at the completion of the infusion (Table I). This parallelism was also evident during period B of experiments 4 through 7, but marked discrepancies are obvious in experiments 1, 2, and 3. When the concentration of serum phosphate fell, its excretion increased and the estimated rate of liberation of the ion from body cells usually decreased. A net movement of phosphate into cells could be demonstrated in seven of the 14 balance periods (Table II). But the rate of release of phosphate from cells appeared to increase (above that of the control period) during six of the balance periods despite accelerated utili-

zation of glucose. Accelerated utilization and storage of carbohydrate, therefore, does not necessarily provoke net cellular retention of phosphate. For example, during certain periods of increased utilization of glucose (Experiments 1–B, 3–B, 4–B, Table II), the concentration of serum phosphate rose despite augmented excretion of the ion. Since contraction of extracellular volume did not fully account for this rise in concentration, it may have been related, in part, to transfer of phosphate from the cellular phase. That the net movement of phosphorus may be away from cells is not, of course, evidence of a failure of participation of inorganic phosphate in carbohydrate metabolism, since striking alterations of the distribution of intracellular components may take place without necessary reflection in overall measurements.

DISCUSSION

The failure of rapid injections of large quantities of hypertonic glucose to provoke appreciable depletion of augmented stores of water and salt indicated that such injections were of little or no value in the treatment of the edema of these patients. This is in accord with other observations (12, 13), although detailed studies of rapid administration of comparable amounts of hypertonic glucose in edema have not been reported previously. It seems probable that the paucity of such studies is at least in part a reflection of a prevalent fear that patients with edema, particularly if it is secondary to heart failure, withstand parenteral fluids poorly as a result of a predisposition to pulmonary edema under these circumstances. Despite extremely rapid expansion of plasma and extracellular volume, one transient episode of increased dyspnea (in a cirrhotic) was the sole respiratory complication. Although solutions of sodium salts might not have been so innocuous under the same conditions, the present study indicated that certain fluids could be given parenterally to edematous patients with greater safety than is sometimes assumed.

Cellular dehydration contributed considerably to the expansion of extracellular volume induced by the infusions of glucose (Table II). This transfer of water from cells could not have occurred if glucose traversed cellular membranes freely, because it would then have elevated the osmotic pressure on both sides of the membrane equally and could not have affected the distribution of water. Hence, free glucose must be largely confined to the extracellular fluid, either because it diffuses into cells slowly or because its penetration is not by diffusion but by an active process involving its metabolic transformation within the cell. This latter would seem the more likely alternative, since not only have direct analyses of skeletal and heart muscles in animals and man demonstrated that free glucose is largely confined to the fraction of muscle water which is extracellular (14, 15), but radioactive glucose has a volume of distribution which seems to approximate extracellular volume (16).

Changes in the volume of the extracellular fluid and the concentration of serum sodium in edematous patients were similar to those noted in normal subjects following similar glucose loads (1). But the renal response to these distortions of the internal environment differed markedly in the two groups (Table I, Figure 1). In normal subjects sodium excretion increased two- to eight-fold during massive glycosuria. In five of seven edematous patients reported here, although the *relative* increase in sodium excretion was far greater than in normal subjects, the *absolute* amounts of sodium lost were trivial because the excretion of sodium initially was negligible. A massive glycosuria of 205 mM/hr. in the case of A. B., although increasing sodium excretion 50-fold, resulted in an excretion of only 1.0 mEq./hr. because the initial rate was 0.02 mEq./hr.

Since measurements of clearances of mannitol and of para-aminohippurate were at about the normal range in the two patients (A. B. and T. L.) in whom salt retention was most tenacious, it is difficult to ascribe such abnormalities of sodium excretion to gross disturbances in renal hemodynamics (7). Nor was there some intrinsic abnormality in the kidneys of the edematous patients which precluded the salt diuresis usually elicited by glycosuria: when circumstances promoting salt retention were altered by bed rest and digitalis (L.), then glycosuria swept salt into the urine in large amounts, as in normal subjects (Figure 1). Initial hyponatremia and antecedent intake low in sodium do not appear to account for the failure of glucose to sweep out large quantities of salt in these patients as contrasted to normal subjects (1). In the latter, striking diminution in concentration of serum sodium after administration of

glucose did not prevent excretion of relatively large quantities of sodium. Moreover, antecedent salt-free diets do not, in normal subjects, prevent the diuresis of salt (17). And finally, marked augmentation of salt excretion is observed during diabetic acidosis when analogous glucose loads are present in patients who are free of edema, but whose intake of salt has necessarily been curtailed (18). Nevertheless, the data suggest that the renal response to administered glucose is not necessarily a function of the filtered glucose or sodium, but varies with those influences in the internal environment which condition tubular activity (19, 20).

The accelerated urinary losses of salt accompanying glycosuria have frequently been considered a purely passive consequence of the osmotic pressure of the nonreabsorbed solute within the renal tubule. The fraction of filtered glucose which is excreted in the urine, by restraining the reabsorption of water in the proximal tubules, is thought to augment the excretion of salt, either by accelerating its passage through the tubules (21, 22) or by diluting the concentration of tubular sodium below that of extracellular fluid (4, 5, 23). The failure of a massive glycosuria, in absolute terms, significantly to augment sodium excretion in these patients militates strongly against any rigid interpretation of this hypothesis. If the capacity of the tubular system for sodium transport were fixed, then such a hypothesis could not account for the negligible losses of sodium observed in most of the patients reported here. On the other hand, if the capacity for tubular transport is variable, largely conditioned by factors in the internal environment, then the concept of a Tm for sodium loses much of its meaning. This does not, of course, imply that there is no intrinsic tubular mechanism for sodium transport which may be influenced by the character and flow of tubular urine, as has been pointed out (22, 24). However, such observations as the failure of a sulfate diuresis to sweep out sodium chloride (25, 26), the variable response to urea loads (1, 2, 6, 23), and the cutback in sodium excretion in some diabetic patients despite continued massive glycosuria (18, 27, 28), suggest that the osmotic influence of the injected solute within the renal tubule may be only one of several determinants. This view is, on the whole, consonant with that expressed in greater detail by Wolf (29).

In Figures 2 and 3 the excretion rates of glucose and of total solutes are plotted against urine flow, both for the patients of this study and for the normal subjects of a previous report (1). The excretion of water per unit of excreted glucose or total solutes was considerably increased by hydration in the normal subject, but was slightly, if at all, changed by hydration in our edematous patients. This undoubtedly reflected the usual difficulty in obtaining a water diuresis in edematous patients. Among the *hydropenic* normal and edematous subjects, the excretion of water per unit of excreted glucose was about the same for both groups. Although there is a suggestion that hydropenic edematous patients excrete slightly more water per mole of total urinary solutes than hydropenic normal subjects, there is too much overlap in the two groups to permit this conclusion.

The accelerated excretion of potassium in the face of declining serum values in the hydropenic subjects of this report has been observed under similar conditions in normal subjects and may have been a result of cellular dehydration, in keeping with previously expressed views on this subject (1, 30–32). The precise nature of the relationship of dehydration to excretion of potassium is, however, obscure. But the results in the hydrated patients, in each of whom there was an overall positive balance of total body water, as well as accelerated excretion of potassium, confirm the emphasis placed on *intracellular dehydration* as a salient determinant of this urinary loss of potassium. The fact that excretion of potassium was not increased in hydrated normal subjects (1), but was augmented in hydrated edematous patients, is unexplained.

The cause of severe, painful muscular spasm which developed in the hydropenic patients about one hour after cessation of the infusion and persisted for several hours is not clear. Since symptoms and signs did not appear in hydrated patients, and ingestion of water probably did in some measure prevent cellular dehydration, the latter may have been a prominent factor in the genesis of these symptoms. If this is the case, edematous subjects must have an additional obscure peculiarity in this respect, since comparable injections of glucose failed to evoke these complications in normal dehydrated subjects (1). Nor does the temporal

sequence provide clear-cut support of this hypothesis, since symptoms were most intense when hyperglycemia had receded and the concentration of serum sodium had been restored. There were no clinical features of tetany and the serum calcium of A. B., although slightly depressed to 7.21 mg. % at the end of the glucose infusion, after an initial value of 8.59 mg. %, subsequently rose to 8.52 mg. % when symptoms had appeared. Nor can these spasms be reasonably attributed to disturbances in metabolism of other electrolytes studied during these experiments. The cramps resembled those described in the condition known as heat cramps (33) or cramps developing during salt depletion when there is free access to water (34). Such cramps have been related to relative intracellular overhydration in conjunction with salt depletion and replacement by water without salt (33). In the present study, however, the concomitant changes in salt and water metabolism differed significantly: There was no remotely comparable depletion of salt and water; there was overexpansion of the extracellular phase and intracellular dehydration; the cramps were obviated by ingestion of water.

It may be significant that the five patients with initially diminished concentrations of serum sodium, and consequent hypotonicity, excreted far less sodium following administration of glucose than did the other two patients (J. W. and L.) in whom the concentration of serum sodium was normal. A similar observation has been made in a study of the retention of administered hypertonic saline by cirrhotics with ascites (7), and is in keeping with the concept (35, 36) that abnormal reduction of the osmotic pressure of body fluids and diminution of the concentration of serum sodium are important features of intractable edema. On the other hand the initially normal values of serum sodium and of osmolar concentration in J. W. and L. may have been the consequence of a prior diuresis induced by other factors (see Procedure). The data suggest that a normal concentration of serum sodium as well as a normal osmotic pressure may be related to the initiation and maintenance of a diuretic response.

SUMMARY

1. Six hundred to 1200 cc. of 25% glucose were administered intravenously over 30 to 90 minutes to three patients with cardiac edema, and four with edema caused by hepatic cirrhosis. Water was given by mouth to four subjects and withheld from three.

2. Urine flow was greatly augmented but losses of water did not exceed the volume of fluid administered. Excretion of sodium, potassium, phosphorus, and chloride usually rose temporarily, as massive glycosuria developed, and then fell. The diuresis of sodium, however, was usually too small to produce appreciable reduction of increased stores of sodium.

3. Concentrations of serum sodium diminished sharply by as much as 20 mEq./L and subsequently returned toward control values. This diminution was apparently produced by extracellular expansion resulting from cellular dehydration attributable to hyperglycemia and attendant increase of effective extracellular osmotic pressure.

4. Serum potassium fell progressively, by as much as 2.2 mEq./L in five patients during initial expansion and despite later contraction of extracellular volume (chloride space). This fall was frequently associated with apparent transfer of potassium into cells, presumably in relation to accelerated utilization and storage of carbohydrate.

5. Aside from increased dyspnea in one patient, severe muscular cramps were the sole complication observed. These occurred exclusively in the patients given glucose without supplementary intake of water and could not be definitely ascribed to changes in concentration of serum electrolytes or to the state of cellular hydration.

6. Findings are discussed in relation to current theories of the nature of "osmotic" diuresis and of the formation of edema.

7. The development of muscular spasms together with the trivial net losses of water and salt suggest that glucose, and probably similar loading substances as well, are of little value in the treatment of intractable edema.

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FAMILIAL DIFFERENCES IN THE PROPORTION OF ABNORMAL HEMOGLOBIN PRESENT IN THE SICKLE CELL TRAIT [1]

By JAMES V. NEEL, IBERT C. WELLS [2] AND HARVEY A. ITANO [3]

(From the Heredity Clinic, University of Michigan, Ann Arbor, Michigan, and the Gates and Crellin Laboratories of Chemistry,[4] California Institute of Technology, Pasadena, California)

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It has recently been shown that the sickling phenomenon exhibited by the erythrocytes of approximately 10 per cent of American Negroes is associated with, and presumably due to, the presence of an abnormal type of hemoglobin molecule (1). In 42 individuals who had only the sickle cell trait, the proportion of abnormal hemoglobin present, as studied by the techniques of electrophoresis, was found to vary from 24 to 45 per cent. In individuals with sickle cell anemia, on the other hand, the proportion of abnormal hemoglobin was usually 100 per cent, although occasionally a small amount of normal hemoglobin was present (1, 2). The sickling phenomenon has been known for many years to have a genetic basis (3), although the exact relationship between the sickle cell trait and full blown sickle cell disease has remained obscure. Recent studies have suggested that the sickling trait and sickle cell disease are related as heterozygote to homozygote, *i.e.*, that there is a gene which when present in single dose results in the sickle cell trait, and when present in double dose, in sickle cell anemia (4, 5). In other words, there exists, primarily in Negro populations, a gene which when represented once in an individual's genetic complex is capable of directing the synthesis of 24 to 45 per cent of the total hemoglobin value along abnormal paths, and when represented twice, results in 85 to 100 per cent of the hemoglobin being of this abnormal type. It has further been shown that although this genetic explanation is adequate to account for the great majority of cases of sickle cell disease, a condition which very closely resembles sickle cell disease, and which may in some instances be clinically indistinguishable from it, may be produced either by the interaction of a single sickle cell gene with the gene responsible for thalassemia, or by the interaction of a sickle cell gene with a newly described gene also responsible for certain electrophoretic abnormalities in hemoglobin behavior (6–8).

The factors responsible for the variation in the proportion of abnormal hemoglobin present in individuals with the sickle cell trait have been unknown. In five sicklemic persons restudied after intervals of two to four months the proportion of abnormal hemoglobin agreed within the limits of experimental error with the original observation, and it is presumed that the proportion of abnormal hemoglobin remains relatively constant in each individual with the sickle cell trait (2). The present studies have been undertaken in an effort to determine to what extent the observed variations in the amount of abnormal hemoglobin present are related to familial factors. More specifically, in a series of families in which the sickle cell trait is present, will there be significant differences from one family to the next in the proportion of the hemoglobin which is abnormal? The existence of such differences could provide one basis for the clinically observed variations in the severity of sickle cell disease from one family to another.

TECHNIQUES

The present investigation is concerned with seven families in which the sickle cell trait was present. Each of these families was selected for study because of its large size and the availability of both parents. In order to establish the diagnosis of sickle cell trait as vs. sickle cell anemia, the individuals included in the study were subjected to the following determinations: presence or absence of sickling, erythrocytes per cmm., grams per cent of hemoglobin, hematocrit, leucocytes per cmm., differ-

[1] This investigation has been aided by grants from the U. S. Public Health Service.

[2] Postdoctoral Fellow of the Division of Medical Sciences of the National Research Council. Present address, College of Medicine, State University of New York, Syracuse, New York.

[3] Assigned to the California Institute of Technology by the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency.

[4] Contribution No. 1581.

ential leucocyte count, and, where indicated, serum bilirubin determinations and reticulocyte counts. The methods of testing for sickling have been described previously (5). On individuals found to sickle, the proportion of abnormal hemoglobin present was determined according to the methods described by Pauling and associates (1) and Wells and Itano (2). For each family it is known that only one parent exhibited the sickle cell trait, the other parent being normal in this respect. This was an essential prerequisite to a study of this nature, since in the event the value observed in a child departed from that found in one parent, it was necessary to know that it was not from the other parent that the child had inherited the gene for sickling. Serological studies involving the A–B–O, M–N, and Rh blood types were done routinely, as a partial check on paternity.

RESULTS

The findings are summarized in Figure 1. On two of the families included in the study (*Hi* and *St*), electrophoretic studies were carried out on the non-sickling as well as the sickling members of the families. This was done for the following reason: In a marriage of a sickler with a non-sickler, a ratio among the children of one sickler: one non-sickler is to be expected if the above outlined genetic hypothesis is correct. In a previous study (5), a slight deficiency of individuals with the sickle cell trait was apparent in the pooled data. One possible explanation of this lay in imperfections of the techniques for eliciting sickling, *i.e.*, the failure of present techniques to produce sickling in all individuals who were genetically predisposed to sickle. Were this so, then one might expect to find the abnormal hemoglobin component present occasionally in individuals who failed to sickle. In the *Hi* and *St* families, there is apparent a deficiency of sickling children. Thus, out of 11 children tested (exclusive of the index cases), only one sickled. However, none of the 10 non-sickling children was found to possess abnormal hemoglobin. The evidence, as far as it goes, thus suggests that the deficiency of individuals with the sickle cell trait is not due to technical failures.

From Figure 1 it is apparent that although in

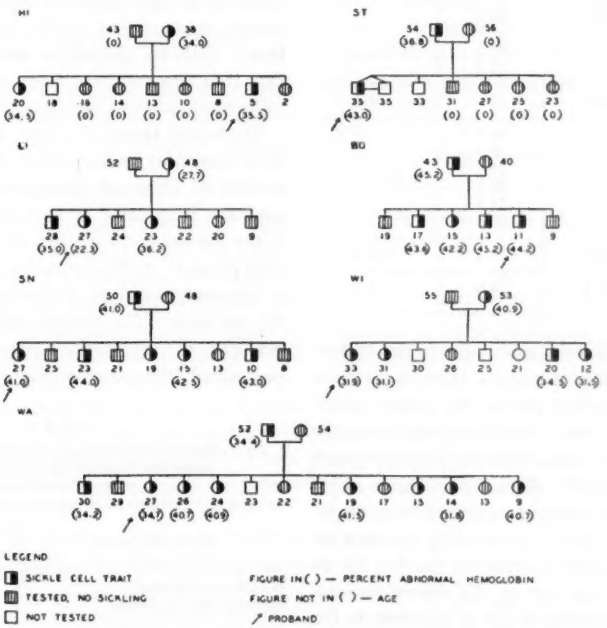

FIG. 1. PEDIGREES OF THE SEVEN FAMILIES STUDIED, TOGETHER WITH DATA ON THE PERCENTAGE OF ABNORMAL HEMOGLOBIN PRESENT IN THE SICKLING MEMBERS OF THESE SEVEN FAMILIES

TABLE I

The relation between sex and the percentage of abnormal hemoglobin present in individuals with the sickle cell trait

Kindred	Males	Females	Difference (Male average minus female average)
Hi	35.5	34.0 34.5	
Av.	35.5	34.3	1.2
Li	35.0	27.7 22.3 36.2	
Av.	35.0	28.7	6.3
Bo	45.2 43.6 45.2 44.2	42.2	
Av.	44.6	42.2	2.4
Sn	41.0 44.0 43.0	41.0 42.5	
Av.	42.7	41.8	0.9
Wi	34.5	40.9 31.9 31.1 31.5	
Av.	34.5	33.9	0.6
Wa	34.4 34.2	34.7 40.7 40.9 41.5 31.8 40.7	
Av.	34.3	38.4	−4.1
All averages	37.8	36.6	1.2

general there are familial similarities in the amount of abnormal hemoglobin present, there may also be rather wide differences among the various members of a family. Some families exhibit a notable constancy (*Hi, Bo, Sn*), whereas others are much more variable (*Li, Wi, Wa*). The degree of intra-family similarity may be measured by an analysis of variance. Before proceeding to such an analysis, it is necessary to examine the data for an influence of age and sex on the determinations. No significant influence of age is apparent in the data. The situation with regard to sex is somewhat different. It can be seen from Table I that in five of the six families which contain sickling individuals of both sexes, the male member or members exhibit a higher average proportion of abnormal hemoglobin than do the females. In the 40 unrelated individuals with the sickle cell trait studied earlier by Wells and Itano (2), a similar tendency is apparent, the average amount of abnormal hemoglobin present in 16 males with the trait being 39.0 per cent, whereas in 24 females it was 37.3 per cent. It follows from this that, other things being equal, families in which the affected members were all of the same sex, or predominantly of the same sex, would be expected to exhibit less variability in the proportion of abnormal hemoglobin than would those where there was near equality as regards the sexes. However, the effect of sex appears to be small as compared to the total variability (and may actually disappear with the accumulation of further material), and no attempt will be made to correct for it in these limited data.

The simplest method of testing for the significance of the differences observed from one family to the next involves an analysis of the total variance observed in these determinations into two fractions, namely, that between families, and that within families. Such an analysis is summarized in Table II. It is apparent that there are very significant differences between family means.

Wells and Itano (2) drew attention to a possible bimodality in the values obtained for the proportion of abnormal hemoglobin. A similar bimodality is apparent from these data. In Figure 2 the combined data from the two studies have been plotted. A clear tendency towards two modes is apparent, one at 34–36 per cent, and one at 40–42 per cent. The relative heights of these two peaks are not necessarily representative of those to be derived from an extensive analysis of the sick-

TABLE II

An analysis of the variation observed in the percentage of abnormal hemoglobin in individuals with the sickle cell trait

Source of variation	Degrees of freedom	Sum of squares	Mean square
All determinations	31	972.52	
Between family means	6	635.73	105.96
Between members of same family	25	336.79	13.47

$$F = \frac{105.96}{13.47} = 7.87 \qquad P < 1\%$$

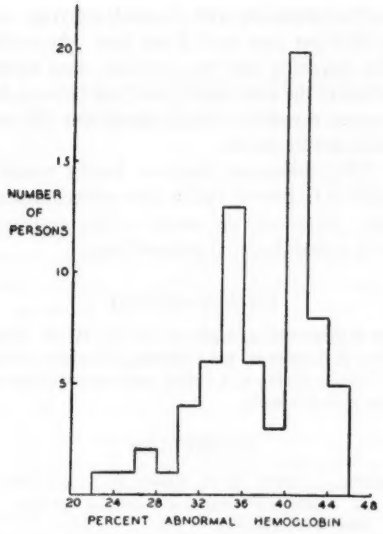

FIG. 2. FREQUENCY HISTOGRAM WITH RESPECT TO THE PERCENTAGE OF ABNORMAL HEMOGLOBIN PRESENT IN THE SICKLE CELL TRAIT

Combined data of this study and Wells and Itano (2).

ling population as a whole, inasmuch as the inclusion of family data when the total number of determinations is so low may have biased the findings.

DISCUSSION

The present data demonstrate clear cut differences among certain families in which the sickling phenomenon is present with respect to the proportion of the hemoglobin which is abnormal in the sickling members of these families. In a number of instances the families studied were widely scattered, and the individuals involved were living under rather diverse circumstances. In the absence of any evidence for significant "environmental" influences on the proportion of abnormal hemoglobin, these differences are presumed to be genetic in origin.

The nature of the genetic mechanism which is assumed to be responsible for these differences is not at present clear. The finding of two modal values with respect to the proportion of abnormal hemoglobin raises the possibility of a genetic dichotomy, such as might be attributed to the existence of two different genes capable of producing the sickling phenomenon, one tending to produce 34–36 per cent abnormal hemoglobin, and the other 40–42 per cent, with the effects of both genes subject to various modifying influences. However, a scrutiny of the pedigrees in Figure 1 raises doubts as to the validity of this interpretation, which would require that the values observed in the sickling children would agree rather closely with those in the sickling parent. While this is true in some families (*Hi, Bo, Sn*), in the *Wi* family a parent with 40.9 per cent abnormal hemoglobin has four sickling children in whom the values range between 31.1 and 34.5 per cent, and in the *Wa* family a parent with 34.4 per cent has seven sickling children, three with values between 31.8 and 34.7 per cent, and four with values between 40.7 and 41.5 per cent. This finding suggests that there may be segregating, in American Negro populations, genes capable of influencing significantly the proportion of abnormal hemoglobin present in individuals homozygous or heterozygous for the sickling gene. By way of illustration, it could be postulated that there is a recessive modifying gene (*m*) capable when homozygous of significantly decreasing the proportion of abnormal hemoglobin produced by a single principal gene responsible for the sickling phenomenon. The results observed in the *Wi* family could be explained thus, where *Sk* represents the gene responsible for the sickling phenomenon, and *sk* its normal allele:

Father (high)		Mother
Sk sk M m	×	*sk sk m m* or *sk sk M m*
↓		
Sk sk m m (low)		

The four sickling children in the *Wi* family with values significantly lower than their father may be assumed to have received the recessive modifier from both parents. Such a marriage should also produce equal or greater numbers of "high" children like the father; it must be further postulated that by chance none has occurred in this marriage. In the *Wa* family the results may be explained as follows:

Father (low)		Mother	
Sk sk m m	×	*sk sk M m*	
↓			
Sk sk m m (low)	and	*Sk sk M m*	(high)

Here the approximately equal numbers of "high"

and "low" children which one might expect from the theory are present.

Wells and Itano (2) were able to study both parents of two of their anomalous cases of sickle cell anemia with small amounts of normal hemoglobin. In one of these cases, the father had 41.0 per cent abnormal hemoglobin and the mother, 40.5 per cent. In this instance both parents could have been heterozygous for the recessive modifier which has been postulated, the child then being homozygous for the modifier.

It should be emphasized that the above scheme is supplied only to illustrate how genetic modifiers could be responsible for the observed results. It would be premature to postulate that this is in fact the actual situation. At this stage in the analysis it does not appear that we are justified in reaching any more definite conclusions than that:

1. There *may* exist sickle cell genes which differ in the proportion of abnormal hemoglobin for which they are characteristically responsible.

2. There *do* exist influences, probably at least in part genetic, which are capable of modifying the proportion of abnormal hemoglobin present in an individual heterozygous for the sickling gene.

SUMMARY

1. Seven Negro families in which the sickle cell trait is present have been investigated by the techniques of electrophoresis with reference to the question of whether there are significant differences between families as regards the proportion of abnormal hemoglobin associated with the trait. These seven families include a total of 74 individuals, of whom 32 are known to have the sickle cell trait and were studied.

2. The proportion of abnormal hemoglobin observed in individuals with the sickle cell trait varied from 22.3 per cent to 45.2 per cent. An analysis of this variation into two portions, that between members of the same family, and that between family means, revealed a highly significant difference between family means.

3. This difference between family means is thought to be genetic rather than environmental in origin. However, the nature of the responsible genetic system is not at present clear.

ACKNOWLEDGMENT

The authors are most grateful to Dr. W. W. Zuelzer and Dr. E. Kaplan of the Children's Hospital of Michigan, Detroit, Michigan, for their many courtesies in connection with this study.

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MULTIPLE MYELOMA. IV. ABNORMAL SERUM COMPONENTS AND BENCE JONES PROTEIN[1]

By R. WAYNE RUNDLES, GERALD R. COOPER, AND ROBERT W. WILLETT[2]

(From the Department of Medicine, Duke University School of Medicine and the Hematology Laboratory, Duke Hospital, Durham, N. C.)

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The overgrowth of plasma cells in multiple myeloma is associated with a unique abnormality in the synthesis of protein. Abnormal components occur in the sera of patients with this disease, sometimes in sufficient amount to produce hyperproteinemia, and the majority excrete Bence Jones protein in the urine.

The relation between plasma cell proliferation and the production of the abnormal proteins, and the relation of the serum components to Bence Jones protein have been obscure. The degree of hyperproteinemia and the amount of Bence Jones protein excreted in the urine vary greatly in patients who appear to have equally progressive and anatomically extensive disease. Large amounts of abnormal protein have been present in the sera of patients with, as well as without, Bence Jones proteinuria, and vice versa. The anomalous serum and urinary proteins vary from patient to patient in electrophoretic mobility, solubility in salt solution, molecular weight and antigenicity (1–11).

It has been suggested that the serum increments in multiple myeloma, especially those with gamma globulin characteristics, may represent antibodies to Bence Jones protein (12). On the other hand, the serum components, which are produced in all likelihood by the abnormal plasma cells (9, 13) may represent a perversion in the normal processes of globulin production (14–16). As to the origin of Bence Jones proteins, compounds of such relatively small molecular size are readily excreted by the kidney (17, 18). Large concentrations have not been found in the serum (5). Bence Jones proteins could possibly be derived from the abnormal serum constituents, since the latter have a molecular size about three to six times as great (3, 5, 12), if the latter were to disintegrate or be split into protein moities filterable through the glomeruli. An additional possibility should not be overlooked, namely, that the proliferating plasma cells might produce more than one type of abnormal protein.

A series of 30 patients with multiple myeloma have been studied, many of them as the activity of their disease was suppressed by therapy and later as relapse occurred, in the investigation of these problems.

MATERIALS AND METHODS

Sera were collected and studied by electrophoresis using barbital buffer at pH 8.6 as previously described (9, 19).

Urine specimens were tested for the presence of protein with 20% sulfosalicylic acid. When proteinuria was present, 24-hour collections of urine were made under toluene at room temperature and the specimens stored at 4° C. The total protein content was determined by the precipitation method previously described (9). Qualitative tests for Bence Jones protein included *1*) testing the protein at a concentration of 0.1–0.4% for coagulation below 60° C. at pH 5.5 in urine or, after electrophoretic separation, diluted in 0.9% NaCl solution, and *2*) testing for solubility at 100° C. followed by reprecipitation on cooling. To demonstrate solubility of the protein at the boiling temperature further acidification with acetic or nitric acid was usually necessary. To purify and concentrate urinary proteins for physico-chemical studies, the refrigerated urine specimens were decanted to remove inorganic precipitates and pooled into suitable quantities. The pH was adjusted to 5.5 with acetic acid and ammonium sulfate slowly added to a final concentration of 60% saturation. The supernatant fluids gave negative tests for protein with sulfosalicylic acid after the salt precipitation. The protein precipitate was separated off by centrifugation and dialyzed at 5–8° C. against water until essentially free of ammonium ion. The protein solutions were stored in a frozen state if not used at once. Electrophoretic analyses of the urine proteins were carried out as for the serum specimens.

Diffusion studies of the serum and urinary proteins were made in the Tiselius apparatus in which boundaries were recorded by the method of crossed slits described by Svensson. At the conclusion of the electrophoresis runs, if large peaks of suitable dimension were present, as in

[1] Supported by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] Damon Runyon Clinical Research Fellow.

Figure 1, the current was turned off and photographs taken at intervals of six to 12 hours. A minor degree of boundary asymmetry was sometimes present. The photographs were enlarged and drawn. The peak boundaries and base lines were drawn through the vertical midpoints of the Philpot-Svensson patterns.

Three formulae, incorporating the maximum ordinate and the maximum ordinate-area methods, were used for calculating diffusion rates (20).

$$(1) \qquad D = F\frac{\mu^2}{2t}$$

$$(2) \qquad D = F\frac{A^2}{4\pi Hm^2 t}$$

$$(3) \qquad D = F\frac{A\mu}{\sqrt{8}\pi Hmt}$$

where

t = time of diffusion in seconds

Hm = maximum ordinate of peak

μ = one-half the horizontal distance between the points of inflection of the vertical sides of the peak, equal to $\frac{Hm}{\sqrt{e}}$ or $\frac{Hm}{1.649}$

$$F = \left(\frac{1}{\text{Magnification}}\right)^2 = \left[\frac{1}{\text{Magnification of Tiselius apparatus}} \times \frac{1}{\text{Magnification of projector for drawing}}\right]^2$$

A = area of peak determined by standardized planimeter.

Diffusion rates were calculated from the average of the three methods for both the ascending and descending sides of the electrophoretically separated protein components. The two values were averaged.

$D_{20,w}$ was calculated by correcting the observed diffusion rate for the influence of temperature and the relative viscosity of the barbital buffer, 1.065 (Neurath [20], equation 46, p. 384).

$D^{\circ}_{20,w}$ was calculated by correcting for the influence of protein concentration on diffusion rate. This correction was made for each individual component by multiplying $D_{20,w}$ by the relative viscosity of the average protein concentration on the two sides of the diffusing peak (see Neurath [20], equation 48). The protein concentration data were available from the preceding electrophoretic analysis. The specific viscosity of albumin was assumed to be 0.04, globulin 0.06, serum containing over 40% albumin 0.05, and serum with albumin less than 40% 0.06 (Neurath, Cooper and Erickson [21]). The diffusion of sucrose in a 2% solution in barbital buffer at pH 8.6 on two occasions were $D_{1^\circ,w} = 24.1$ and 22.7, compared to the average value determined by Gosting and Morris (22) of 23.83 ($\times 10^{-7}$). The diffusion of purified beef serum albumin (Armour) on three occasions was $D^{\circ}_{20,w} = 6.4$, 6.5 and 6.0. The average of 13 calculations of the diffusion of human albumin in the sera studied was $D^{\circ}_{20,w} = 6.8 \pm 0.4$.

After completing the electrophoretic and diffusion measurements, the buffer solution was removed from the descending side of the Tiselius cell above the peak representing the abnormal gamma or "M" component. The latter was then removed under visual control and studied in the ultracentrifuge using the same buffer as employed in electrophoresis and diffusion. The sedimentation analyses were performed with the electrically driven, Model E, Ultracentrifuge built by the Specialized Instruments Corporation, as described by Schwert (23). Boundary displacements were measured by enlargement of the Philpot-Svensson photographs. The log of the distance from the center of rotor (x) was plotted against time in seconds (t). The uncorrected sedimentation constant (S_t) was calculated from

$$S_t = \frac{dx/dt}{\omega^2 x}$$

or on integration

$$S_t = 2.3026\frac{\Delta \log x}{\omega^2 \Delta t}$$

where ω is speed in radians/sec. and dx/dt is cm./sec.

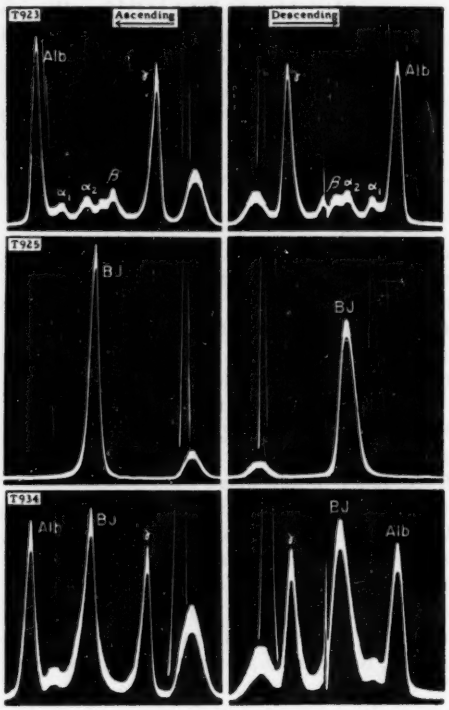

FIG. 1. CASE 1, A. R., C19679. PHOTOGRAPHS OF ELECTROPHORETIC PATTERNS OF SERUM AND URINARY PROTEINS

T923, serum during relapse; T925, Bence Jones protein from urine; T934, mixture of serum and urinary protein.

The sedimentation constant in water at 20° C. ($S_{20,w}$) was calculated by the method of Svedberg and Pedersen (24) (eq. 63, p. 36). The relative viscosity of the barbital buffer used was 1.065. The specific molal volume of the protein was taken to be 0.75 in all cases. The density of the barbital buffer at 30° C. was 0.0079 above water.

$S^{\circ}_{20,w}$ was computed as suggested by Lauffer (25) by multiplying $S_{20,w}$ by the relative viscosity of the protein solution. The latter was taken as 1 plus the specific viscosity of the protein component multiplied by protein concentration.

The molecular weight of the proteins was computed from the sedimentation and diffusion values as follows (24):

$$\text{Mol. wgt.} = \frac{RTS_{20,w}}{D_{20,w}(1 - V\rho_{20,w})} = 9.696 \times 10^4 \times \frac{S_{20,w}}{D_{20,w}}$$

where

R = gas constant, 8.313×10^7
T = absolute temperature
V = assumed to be 0.750
$\rho_{20,w}$ = density of water at 20° C. = 0.9982

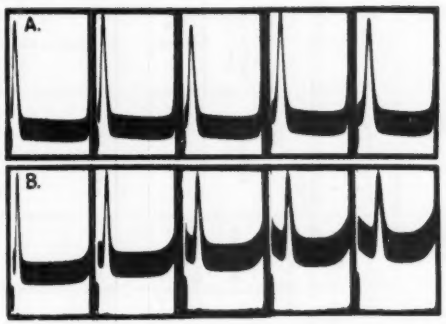

FIG. 2. CASE 1, A. R., C19679. PHOTOGRAPHS AT EIGHT MINUTE INTERVALS OF ULTRACENTRIFUGE SEDIMENTATION BOUNDARIES

A, Bence Jones urinary protein; B, gamma protein separated after electrophoresis (T923, Figure 1).

OBSERVATIONS

The major physico-chemical data relating to the abnormal serum and urinary proteins in the individual patients with multiple myeloma are given in Table I. Their correlation with the severity and anatomic extent of the myelomatous disease, renal function, and other relevant data is summarized below. Additional clinical and therapeutic information in some cases has been reported elsewhere, as indicated.

Case 1. A. R., C19679. This patient was observed over a period of more than three years (9, 26, 27). Her serum contained an abnormal component of gamma mobility that fluctuated from 4.0 to 1.4 gm. per 100 cc. as the activity of her disease was modified by urethane therapy. During a relapse 18½ months after the initial treatment, the gamma protein concentration in her serum was 3.0 gm. per 100 cc. (Table I, T745). The Bence Jones protein excretion at this time was 2–3 gm. per day. Four months later the serum component was reduced to 1.4 gm. per 100 cc. and the proteinuria to 0.3–0.5 gm. During subsequent relapses gamma protein increased to 2.7 and 3.6 gm. per 100 cc. and the urinary protein ranged from 6–10 gm. per day. Inulin and p-aminohippurate clearance studies on three occasions showed moderate impairment of renal function (28).

Electrophoretic studies of the protein from pooled urine specimens collected during the ninth and 19th–24th months of observation showed an almost pure homogeneous protein (Figure 1, T925). The mobility of the abnormal serum component was 1.2, that of the protein in the urine, 3.8. To study the effect of different media on electrophoretic mobility, protein from the urine was added to her serum and the electrophoresis repeated. The urinary protein separated from the gamma component of the serum and had the mobility of alpha$_2$ globulin, 2.9 (Figure 1, T934).

The diffusion rate of the gamma component in the serum, measured in the Tiselius apparatus after electrophoresis, was $D^{\circ}_{20,w} = 4.9$. When removed and studied in the ultracentrifuge, it sedimented as one component, $S^{\circ}_{20,w} = 6.6$ (Figure 2,B). The molecular weight was calculated at 130,000. The diffusion and sedimentation rates of the urinary protein were 6.7 and 2.80, respectively, and its calculated molecular weight, 40,300.

Case 7. A. A. P., 59025. This patient had extreme skeletal disease, hypoproteinemia and heavy proteinuria of at least three and one-half years' duration (9). Electrophoretic analysis of serum obtained before therapy, showed a normal pattern except for slight sharpness in the gamma boundary. Bence Jones proteinuria at this time averaged 18–20 gm. per day. Inulin and p-aminohippurate clearance studies on two occasions showed little or no impairment of renal function (28).

The urinary protein was studied electrophoretically on four occasions during a period of 20 months. Over 95% of the protein was represented by a homogeneous component with a mobility of 1.5. When urinary protein was added to the serum, its electrophoretic mobility was the same as that of the abnormal gamma component.

Two diffusion studies of the urinary protein showed $D^{\circ}_{20,w} = 9.1$ and 7.7. The latter value was determined under the more favorable technical circumstances. Three ultracentrifuge runs at protein concentrations of 0.73%, 1.5%, and 2.87% showed a single component, $S^{\circ}_{20,w} = 3.45$, 3.36, and 3.35, respectively. The molecular weight of the urinary protein was calculated at 37,100–42,000 (Table I).

Case 11. J. F., C47338. This 72 year old lawyer had impaired health of three to four years' duration, extensive skeletal disease, and mild anemia (9). The NPN was not elevated but inulin and p-aminohippurate clearances showed severely impaired renal function (28). The serum protein concentration before therapy was increased to 13.0 gm. per 100 cc. of which 9.0 gm. were a gamma component, mobility 1.4. He excreted 1.6–2.2 gm. of protein in the urine per day of which 90% by electrophoretic analysis was a homogeneous component with a mobility of 2.7 (Table I).

TABLE I

Physico-chemical characteristics of abnormal serum components and urinary proteins in multiple myeloma

	Serum protein										Urine protein							
	Tiselius analysis number	Abnormal serum component	Quantity of abn. component		Electrophoretic mobility *cm.²/sec./volt*	Diffusion rate		Sedimentation rate		Calculated molecular weight	Amount excreted *gm./24 hrs.*	Tiselius analysis number	Protein composition	Mobility abn. comp. *cm.²/sec./volt*	Diffusion rate		Sedimentation rate		Calculated molecular weight
			%	*gm./100 cc.*		$D_{20,w}$	$D^{0}_{20,w}$	$S_{20,w}$	$S^{0}_{20,w}$						$D_{20,w}$	$D^{0}_{20,w}$	$S_{20,w}$	$S^{0}_{20,w}$	
Patients with abnormal serum components of gamma mobility																		
Case 1 A. R., C-19679	745 818 854 920 923	Tall, thin gamma peak Low, sharp gamma peak Tall, thin gamma peak Tall, thin gamma peak Same serum as above	34 16 32 37	3.0 1.4 2.7 3.7	1.2 1.2 1.1 1.3	 4.7 4.6	 4.9 4.9	 6.37 6.37	 6.58 6.60	 129,200 130,600	2.2–3.1 0.3–0.5 6.1–8.2 5.8–10.3	925	Single, thin alpha₂ peak	3.8	6.4	6.7	2.51	2.80	40,300
Case 7 A. A. P., 59025	639	Slight gamma sharpness	10	0.5	1.4						18–20 3.0–3.6	902 926	Tall, thin gamma peak Tall, thin gamma peak	1.5	8.9 7.4	9.1 7.7	3.38 3.01 3.17	3.45 3.35 3.36	37,100 42,000
Case 11 J. F., C-47338	668 832 921	Tall, thin gamma peak Tall, thin gamma peak Tall, thin gamma peak	69 46 59	9.0 4.3 5.8	1.4 1.7 1.5	 3.8 4.3	 4.0 4.5	 6.12	 6.43	 138,600	1.6–2.2	788	Tall, "M" peak, 90%	2.7			3.00	3.27	
Case 13 J. R., C-53385	714 839	Slight gamma sharpness Slight gamma sharpness	8 9	0.5 0.6	1.6 1.5			 6.65	 6.76		1.5–2.3	791	Mixture, gamma protein 70%	1.6			3.50	3.78	
Case 14 P. L. F., C-8339	712 742	Tall, thin gamma peak Tall, thin gamma peak	47 47	3.9 3.2	1.0 1.1						None None								
Case 15 L. B., C-54586	820	Low gamma peak	22	0.9	1.5						+++	824	Tall, thin gamma peak	1.6	6.4	6.7	3.28	3.58	51,900
Case 18 J. D., C-60817	763 838	Tall gamma peak Tall gamma peak	42 46	3.8 4.4	1.3 1.3	 4.1	 4.4	 6.30	 6.68	 146,500	0.1–0.25 None								
Case 19 E. W. S., C-61008	837	Low gamma peak	19	1.8	1.5			6.34	6.44		None								
Case 20 B. K. H., C-63673	768 821* 907 914	Tall, thin gamma peak Low, sharp gamma peak Tall, thin gamma peak Tall, thin gamma peak	61 14 58 53	8.4 1.0 6.0 4.9	1.1 1.5 1.2 1.3	 3.4 3.9 4.0	 3.6 4.1 4.2	 6.16 6.30	 6.49 6.57	 153,000 151,700	0.6–0.9 None 1.5–2.0	919	Mixture. 48% gamma Gamma fraction of T919	1.5	8.9	9.2	2.74 3.13	2.96 3.16	31,200 33,300

TABLE I—*Continued*

	Serum protein										Urine protein								
	Tiselius analysis number	Abnormal serum component	Quantity of abn. component		Electrophoretic mobility *cm.²/sec./volt*	Diffusion rate		Sedimentation rate		Calculated molecular weight	Amount excreted *gm./24 hrs.*	Tiselius analysis number	Protein composition	Mobility abn. comp. *cm.²/sec./volt*	Diffusion rate		Sedimentation rate		Calculated molecular weight
			%	*gm./100 cc.*		$D_{20,w}$	$D^{\infty}_{20,w}$	$S_{20,w}$	$S^{\infty}_{20,w}$						$D_{20,w}$	$D^{\infty}_{20,w}$	$S_{20,w}$	$S^{\infty}_{20,w}$	
Patients with abnormal serum components of gamma mobility—*Continued*																			
Case 22 E. Z. F., C-66471	817 913	Low, sharp gamma peak Low, sharp gamma peak	11 17	0.7 1.2	1.8 1.6			6.36	6.45		10–11 10–14	823 896	Tall, thin gamma peak Tall, thin gamma peak	1.6 1.5	6.8 7.2	7.1 7.4	3.21	3.48	45,400
Case 24 J. H. McI., C-70153	836 915	Tall, thin gamma peak Tall, thin gamma peak	60 70	7.9	0.3	3.8	3.9	5.62 6.22	6.26 6.45	159,000	1.6–1.9 1.0–1.3	898	Tall, thin gamma peak 87%	1.2	9.5	9.8	2.28	2.44	24,000
Case 25 W. H. P., C-76005	911	Tall, thin gamma peak	64	7.4	1.0	4.1	4.3	6.46	6.69	152,300	0.05–0.07								
Case 28 J. P. D., C-80716	908	Low, sharp gamma peak	13	1.0	0.9						None								
Case 29 C. C. C., C-84722	909	Low, sharp gamma peak	19	1.5	0.7			6.39	6.52		6.4–7.5	900	Tall, thin gamma peak 92%, albumin 3%	1.6	4.6	4.7	3.98	4.40	90,200
Case 30 H. L. O., C-84862	906	Tall, thin gamma peak	66	8.1	1.0	2.9	3.1	6.19	6.60	205,800	0.2–0.35	924	Mixture. Gamma protein 31%	1.7					
Case 31 J. M. W., C-89590	905	Low, sharp gamma peak	14	1.0	1.6						2.8–4.0	916	Thin, sharp beta peak	2.5	6.7	7.0	3.12	3.44	47,400
Case 35 W. H. K., C-94891	930	Tall, thin gamma peak	43	3.8	0.8	4.0	4.3	6.34	6.67	151,800	None								
Patients with abnormal serum components of "M" mobility																			
Case 4 S. J. C., C-28643	637* 717*	Tall, sharp "M" peak Tall, sharp "M" peak	33 31	2.8 2.5	2.4 2.2						20–22 6.8–10.8	784	Tall, thin alpha₂ peak	4.1	8.5 (pH 5.5)	8.6 (pH 5.5)	3.02	3.27	36,900
	819* 847*	Tall, sharp "M" peak Small "M" peak	27 14	2.3 1.0	2.4 1.9	4.6	4.9				11–14 0.25–0.50								
Case 6 J. C., C-27868	848	Low, sharp "M" peak	18	1.4	1.9						None								

TABLE I—*Continued*

	Serum protein										Urine protein								
	Tiselius analysis number	Abnormal serum component	Quantity of abn. component		Electrophoretic mobility *cm.²/sec./volt*	Diffusion rate		Sedimentation rate		Calculated molecular weight	Amount excreted *gm./24 hrs.*	Tiselius analysis number	Protein composition	Mobility abn. comp. *cm.²/sec./volt*	Diffusion rate		Sedimentation rate		Calculated molecular weight
			%	*gm./100 cc.*		$D_{20,w}$	$D^{0}_{20,w}$	$S_{20,w}$	$S^{0}_{20,w}$						$D_{20,w}$	$D^{0}_{20,w}$	$S_{20,w}$	$S^{0}_{20,w}$	
Patients with abnormal serum components of "M" mobility—*Continued*																			
Case 8 C. K., C-44556	657	Tall, thin "M" peak	71	8.6	2.4						0.3	835	Mixture, alpha₂ protein 70%	4.0					
Case 9 M. J. W., C-35338	667	Small "M" component	5	0.4	2.6						0.2	797	Mixture, beta protein 70%, albumin 30%	3.1					
Case 12 C. M. M., C-51009	689	Tiny "M" component	±2		2.6						1–2	826	Mixture, beta protein 82%	3.0			2.90	3.24	
Case 27 E. P., C-77475	903 910	Tiny "M" component Tiny "M" component	2 2	0.1 0.1	2.3 2.2						0.02–0.1 0.2								
Case 32 B. N., C-90306	899	Tall, thin "M" peak	49	6.1	2.3	4.7	5.0	5.87	6.19	120,300	0.2–0.6	922	Mixture, "M" protein 18%	2.3					
Case 33 M. G. K., C-92011	918	Small "M" component	4	0.3	2.2						4–5	931	Albumin 19%, "M" protein 67%	2.3	5.9	6.0	3.69	3.77	60,700
Patients with normal serum electrophoretic pattern																			
Case 16 W. W. B., C-55643	766	No definite abnormality									0.1–0.6	829	Sharp beta peak, 77%	3.1					
Case 17 S. H., 93158	769	No definite abnormality									1.2	830	Mixture, beta protein 43%, albumin 44%	3.9					
Case 21 L. N., A-33105	775 850	No definite abnormality No definite abnormality									None 0.2–0.8	828	Mixture, albumin 48%, "M" protein 25%	2.7					
Case 26 J. B., C-77930	912	No definite abnormality									0.1–0.2								
Case 34 P. T., C-93777	917	No definite abnormality									None								

* Calculations made from photographs of descending patterns.

In the ultracentrifuge, gamma protein from the serum and the urine protein sedimented as homogeneous components, $S^{\circ}_{20,w} = 6.43$ and 3.27, respectively. The molecular weight of the abnormal serum constituent was calculated at nearly 140,000. The sedimentation rate of the urine protein was comparable to that of Bence Jones protein with a molecular weight of around 40,000.

Case 13. J. R., C53385. This 57 year old housewife, admitted to Duke Hospital on February 3, 1949, had developed, in 1938, a large tumor in the frontal area of the skull that became smaller after irradiation. In 1944, when thoracic vertebrae collapsed, x-ray films showed generalized skeletal demineralization. Five years later she had radiologic evidence of extremely severe skeletal disease, moderately severe anemia, and overgrowth of abnormal plasma cells in the bone marrow. There was no nitrogen retention. Inulin and p-aminohippurate clearances showed severely impaired renal function (28).

Two electrophoretic studies of the serum, one before therapy and another six months later, showed as the only abnormality, slight sharpness in the gamma protein boundary. The gamma component from the second electrophoretic run was removed and studied in the ultracentrifuge. Only a single component was demonstrable, $S^{\circ}_{20,w} = 6.76$.

During her initial hospital stay she excreted 1.5–2.3 gm. of protein per day in the urine. Seventy % of the protein on electrophoretic analysis was a single component with a mobility of 1.6 (Figure 7). The protein mixture was studied in the ultracentrifuge at a concentration of 1.98 gm. per 100 cc. The major component sedimented with a sharp boundary, $S^{\circ}_{20,w} = 3.78$.

Case 14. P. L. F., C8339. This patient, with a plasmacytoma of the scapula, had an abnormal gamma component in her serum in a concentration of 3.9–3.2 gm. per 100 cc. (9, 27). Proteinuria was never detected. Inulin and p-aminohippurate clearance studies showed moderate impairment of renal function (28).

Case 15. L. B., C54586. This 49 year old colored woman was admitted to the hospital with severe congestive failure, anasarca and hypoproteinemia. She died a few hours later (27). There was no nitrogen retention. Proteinuria was heavy but the quantity excreted per day was not determined.

An abnormal gamma component with a mobility of 1.5 was present in the serum in a concentration of 0.9 gm. per 100 cc. Almost all of the urinary protein on electrophoretic analysis was one homogeneous component. Its mobility was approximately that of the gamma increment in the serum. In the ultracentrifuge the urinary protein sedimented as one homogeneous component. From the diffusion and sedimentation measurements its molecular weight was calculated at nearly 52,000.

Case 18. J. D., C60817. This 58 year old colored farmer with extensive skeletal disease and mild anemia had a serum protein concentration of over 9.0 gm. per 100 cc. with an abnormal gamma component of 3.8–4.4 gm. (9) per 100 cc. His urine contained little or no protein. Inulin and p-aminohippurate clearance studies showed moderate impairment of renal function (28).

The gamma protein separated from the serum electrophoretically sedimented in the ultracentrifuge as a single component. The molecular weight was calculated at 146,500.

Case 19. E. W. S., C61008. This 42 year old physician developed, in a period of six months, several areas of myelomatous skeletal destruction. His serum contained an abnormal gamma component amounting to 1.8 gm. per 100 cc. (9, 27). Only minute traces of protein were present in the urine. Clearance studies showed normal renal function (28). Gamma protein, separated from the serum electrophoretically, sedimented in the ultracentrifuge as a single component, $S^{\circ}_{20,w} = 6.44$.

Case 20. B. K. H., C63673. This 28 year old student had been ill with fever and severe anemia for six months. Before therapy his serum contained 13.9 gm. of protein per 100 cc. of which 8.4 gm. were an abnormal gamma component. Proteinuria ranged from 0.6–0.9 gm. per day (9). During a remission of his disease that lasted for several months, the gamma component in the serum fell to 1.0 gm. per 100 cc., and the urine became free of protein. During a fatal exacerbation of the myeloma following meningitis he developed hypercalcemia but had no nitrogen retention. Gamma protein in the serum at this time rose to 6.0–4.9 gm. per 100 cc., and the proteinuria to 1.5–2.0 gm. per day.

Gamma protein was separated from the serum electrophoretically on two occasions and studied in the ultracentrifuge in concentrations of 0.89% and 0.71%. Only a single homogeneous component could be identified, $S^{\circ}_{20,w} = 6.49$ and 6.57. The molecular weight calculated from the diffusion and sedimentation values was about 152,000.

Electrophoretic study of the urinary protein, excreted during the final episode, showed a mixture of which nearly half was a component with virtually the same mobility as that of the abnormal serum constituent (Table I and Figure 7).

The urinary protein mixture was studied in the ultracentrifuge at a concentration of 2.0 gm. per 100 cc. The $S^{\circ}_{20,w}$ of the major urinary component was 2.96. The gamma component of the urinary protein, removed after electrophoresis, sedimented as a homogeneous component, $S^{\circ}_{20,w} = 3.16$. The molecular weight was calculated at about 33,000 (Table I).

At necropsy there was round cell infiltration and some hemorrhage in all the tissues. Round cell infiltration and calcinosis were present in the kidneys but there were no outstanding glomerular or tubular lesions.

Case 22. E. Z. F., C66471. This 57 year old farmer, disabled with back pain for four months, was admitted to the hospital after two to three weeks of rapidly progressive weakness, nausea, vomiting and confusion (27). X-ray films of the skeleton showed demineralization with compression of the 2nd and 4th lumbar vertebrae. The hemoglobin was 8.1 gm. per 100 cc. Bone marrow aspirated from the sternum contained 70% abnormal plasma cells. The blood NPN was 55, serum calcium 13.3 and phosphorus 5.1 mg. per 100 cc. The serum protein concentration was 6.0 gm. per 100 cc. He was given 220 gm. of urethane in a period of two months with only slight benefit.

He died five months after the diagnosis of multiple myeloma was made.

Two electrophoretic analyses of the serum showed an abnormal gamma component with a mobility of 1.8–1.6 present in a concentration of 0.7–1.2 gm. per 100 cc. (Table I). In the ultracentrifuge the gamma protein sedimented as one homogeneous component, $S^{\circ}_{20,w} = 6.45$. Bence Jones proteinuria at the time of the serum analyses ranged from 10–14 gm. per day. Electrophoretic analysis of the urinary protein showed that over 95% of it was a homogeneous component with virtually the same electrophoretic mobility as the abnormal serum increment. In the ultracentrifuge the urine protein sedimented as one component. From the diffusion and sedimentation rates the molecular weight was calculated at 45,400.

Case 24. J. H. McI., C70153. This 55 year old colored laborer had developed severe low back pain with sciatic radiation while lifting freight, two and one-half months before admission. Subsequently his left elbow became swollen and painful. His appetite failed and his weight fell from 147 to 101 lbs. During his last week at home he was confined to bed. Examination showed undernutrition, generalized skeletal tenderness, and a fusiform swelling of his left elbow with local heat, and limitation of movement.

A normochromic, normocytic anemia with hemoglobin reduced to 5.9 gm. per 100 cc. was present. The sternal bone marrow contained 80% abnormal plasma cells. X-ray films of the skeleton showed severe generalized demineralization, without localized osteolytic lesions, and bony destruction about the left elbow with loss of joint space and irregularity of the articular surfaces. The NPN was 68, calcium 11.8 and phosphorus 5.5 mg. per 100 cc. The serum protein concentration varied from 10.8 to 14.0 gm. per 100 cc. The urine contained on an average 1.3 gm. of protein per day that gave the coagulation reactions of Bence Jones protein. A phenolsulfonphthalein test of renal function showed 20% of the dye excreted in one hour and 22% in two hours. Inulin and p-aminohippurate clearance studies showed a glomerular filtration rate of 32.2 cc. per minute, effective renal plasma flow of 309 cc. per minute, and maximal tubular excretion of p-aminohippurate 31 mg. per minute. The filtration fraction was 0.10.

After three weeks of urethane therapy, the NPN and serum calcium values became normal, and the serum globulin concentration was reduced somewhat. After a few weeks he became free of pain, able to walk about and regained the use of his elbow. He remained moderately anemic, however. Four months after his hospital admission he died suddenly at home.

The electrophoretic pattern of his serum before therapy had a tall, sharp peak of very slow mobility, 0.3. When this component was separated from the serum electrophoretically and studied in the ultracentrifuge, it sedimented with a single sharp boundary, $S^{\circ}_{20,w} = 6.26$ and 6.45. The molecular weight calculated from the diffusion and sedimentation rates was 159,000. Electrophoretic study of the urinary protein showed that over 90% of it was a homogeneous component with a mobility of 1.2. When studied in the ultracentrifuge at a concentration of 1.7 gm. per 100 cc., it sedimented with a sharp boundary, $S^{\circ}_{20,w} = 2.44$. The calculated molecular weight was 24,000.

Case 25. W. H. P., C76005. This 69 year old farmer had had severe weakness, anorexia, occasional nausea and vomiting, and pain about his trunk, spine and hips for four to six months. Examination on admission to the hospital showed him to be undernourished, chronically ill and uncomfortable due to skeletal pain. There was pronounced tenderness of all the superficial bones.

Blood studies showed a normochromic, normocytic anemia, with hemoglobin reduced to 9.0 gm., and a leucopenia of 2,500–2,000. Marrow aspirated from the sternum and iliac crest contained 10–20% abnormal plasma cells. X-rays of his skeleton showed generalized demineralization and indefinite areas of osteolytic destruction. The NPN was 35, serum calcium 11.6, and phosphorus 2.8 mg. per 100 cc. The serum protein concentration was 10.2 gm. per 100 cc. The urine contained 0.05–0.07 gm. of protein per day that gave the qualitative reactions of Bence Jones protein. The phenolsulfonphthalein dye was excreted at a normal rate.

He was given 67 gm. of urethane during his 17 days in the hospital but then returned home, contrary to advice, and died three weeks later.

Electrophoretic study of the serum showed a large gamma component with a mobility of 1.0 present in a concentration of 7.4 gm. per 100 cc. The molecular weight calculated from diffusion and sedimentation measurements was about 152,000.

Case 28. J. P. D., C80716. This 29 year old business man developed pain about the right hip of sciatic radiation that gradually became more severe during a period of five months. X-ray films showed a large area of cystic demineralization in the right sacrum and ilium. Small osteolytic lesions were present in two ribs and in the center of the 5th lumbar vertebra. Biopsy of the ilium showed a plasmacytoma. He was given x-ray therapy to the pelvis and obtained partial relief of pain.

Physical examination two months later showed residual tenderness about the right iliac crest. There was no anemia. Bone marrow aspirated from the sternum was virtually normal. The NPN was 30, calcium 9.3, and phosphorus 2.9 mg. per 100 cc. The alkaline phosphatase was 2.8 Bodansky units per 100 cc. The serum protein concentration was 7.9 gm. per 100 cc. The excretion of phenolsulfonphthalein dye was normal. After he had taken 200 gm. of urethane by mouth during a period of two months, his pain subsided and he was able to work full-time.

Electrophoretic analysis of the serum showed an abnormal gamma component, with a mobility of 0.9, present in a concentration of 1.0 gm. per 100 cc. A second analysis 10 months later, after he had taken 1.0–1.5 gm. of urethane daily for six months, showed a reduction in the gamma component to 0.7 gm. per 100 cc. Protein was not found in the urine at any time.

Case 29. C. C. C., C84722. This 50 year old colored farmer complained of gradually increasing pain about his

chest and spine for eight months and loss of 30 lbs. in weight. Three weeks before he was seen, his legs became numb and too weak for him to stand. Examination on admission to the hospital showed generalized bone tenderness, paralysis of the left leg, and impaired cutaneous and vibratory sensibilities up to the knee on the left and to the groin on the right.

Blood studies showed a normochromic, normocytic anemia with hemoglobin reduced to 7.6 gm. per 100 cc. The majority of the cells in marrow aspirated from the sternum were abnormal plasma cells. X-ray films of the skeleton showed osteolytic lesions in the ribs, scapulae, clavicles, humeri, skull, pelvis, proximal humeri and spine with collapse of T5, 8, 10, and 11. The urine contained 6.4–7.5 gm. of Bence Jones protein per day. The NPN was elevated to 57–60 mg.%. The serum calcium was 14.0 mg., phosphorus 4.0 mg., and alkaline phosphatase 3.0 Bodansky units per 100 cc. The serum protein concentration was 7.8 gm. per 100 cc.

After one month of urethane therapy he was free of pain at rest and able to begin moving about. The nitrogen retention and hypercalcemia disappeared. His anemia began to subside in six weeks time and the proteinuria fell to about one-third of the pretreatment level. The neurologic abnormalities disappeared. At five months he was able to walk one and one-half miles to town without discomfort or disability.

Electrophoretic analysis of the serum before treatment showed an abnormal component in the gamma protein, mobility 0.7, and concentration 1.5 gm. per 100 cc. (Figure 3, T909). Gamma protein, separated from the serum electrophoretically, sedimented in the ultracentrifuge with a single sharp boundary, $S^{\circ}_{20,w} = 6.52$.

Electrophoretic study of the urinary protein showed that 92% was a homogeneous component of mobility 1.6 (Figure 3, T900). In the ultracentrifuge it sedimented as one component, $S^{\circ}_{20,w} = 4.40$. Its molecular weight was calculated at about 90,000.

The urinary protein was added to the serum and the electrophoresis repeated. The protein from the urine separated from the abnormal serum component and showed a mobility of 1.3 (Figure 3, T936).

Case 30. H. L. O., C84862. This 66 year old white male was admitted to Duke Hospital after eight to 12 months of increasingly severe back pain. On physical examination he had pronounced tenderness over the ribs, shoulders, spine and pelvis. X-ray films of the skeleton showed osteolytic lesions and demineralization of the ribs, clavicles, humeri and vertebrae, collapse of D10 and L5, and almost complete destruction of L4. Blood studies disclosed a normochromic, normocytic anemia with hemoglobin reduced to 9.6 gm. per 100 cc. Marrow aspirated from the sternum contained over 60% abnormal plasma cells. A small amount of protein was present in the urine, 0.2–0.35 gm. per day, that gave the coagulation reactions of Bence Jones protein. The NPN was 32 mg., calcium 10.2 mg., phosphorus 4.2 mg. and phosphatase 2.2 Bodansky units per 100 cc. The serum appeared viscous. Its protein content was increased to 12.1 gm. per 100 cc. The thymol turbidity test was strongly positive, 30 units of turbidity and 4+ flocculation.

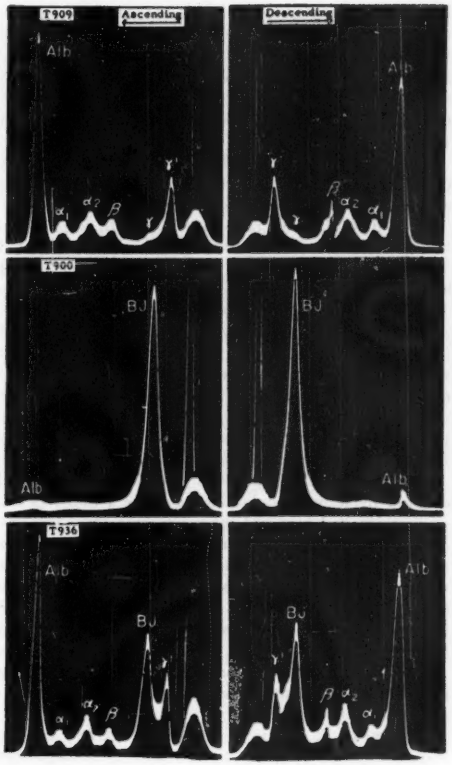

FIG. 3. CASE 29, C. C. C., C84722. PHOTOGRAPHS OF ELECTROPHORETIC PATTERNS OF SERUM AND URINARY PROTEINS

T909, serum before treatment; T900, Bence Jones protein from urine; and T936, mixture of serum and urinary proteins.

He was given 120 gm. of urethane during a period of four weeks in the hospital but he steadily became worse. He was taken home and died two days later.

Electrophoretic analysis of the serum showed an extraordinarily large gamma increment, 8.1 gm. per 100 cc., with a mobility of 1.0. This component, when separated from the serum electrophoretically and studied in the ultracentrifuge, sedimented with one sharp boundary, $S^{\circ}_{20,w} = 6.60$. Its molecular weight was calculated at somewhat over 200,000.

Protein was recovered from a large volume of urine. On electrophoresis it was a mixture in which the largest component was gamma protein with a mobility of 1.7.

Case 31. J. M. W., C89590. This 53 year old white

farmer was admitted to the hospital for the investigation of a painful 8 × 10 cm. tumor of his left chest wall which had developed in a period of two months. Roentgen films showed partial destruction of the 9th to 11th ribs with surrounding soft tissue swelling, and a cystic area 4 cm. in diameter in the body of the pubis on the right. Biopsy of the chest tumor showed it to be a plasmacytoma.

Study of the peripheral blood showed no anemia or cellular abnormality. Marrow aspirated from a tender area in the sternum contained 98% abnormal plasma cells. The blood NPN, serum calcium, phosphorus, and alkaline phosphatase determinations were normal. He excreted 2.8–4.0 gm. of Bence Jones protein in the urine per 24 hours.

Electrophoretic analysis of the serum showed an abnormal gamma component with a mobility of 1.6 present in a concentration of 1.0 gm. per 100 cc. Nearly 100% of the urinary protein on electrophoresis was a homogeneous component of mobility 2.5. It sedimented in the ultracentrifuge as one sharply bounded component. The molecular weight was calculated from diffusion and sedimentation measurements at about 47,000.

Case 35. W. H. K., C94891. This 72 year old retired engineer requested examination regarding a tumor of the skull which had been enlarging for some three months. X-ray films showed a large area of bony destruction under the tumor and numerous small osteolytic lesions, such as were present also in the ribs, vertebrae, and pelvis. Biopsy of the skull tumor showed it to be a plasmacytoma. The peripheral blood findings were normal. Marrow aspirated from the sternum contained 15% abnormal plasma cells. The blood NPN, serum calcium, phosphorus, and alkaline phosphatase determinations were normal. Many urinalyses showed no protein.

Electrophoretic study of the serum showed a large abnormal gamma component with a mobility of 0.8 present in a concentration of 3.8 gm. per 100 cc. When separated off electrophoretically and studied in the ultracentrifuge, it sedimented as one component. The molecular weight was calculated at nearly 152,000.

Case 4. S. J. C., C28643. This patient's illness was studied for a period of over two years (9, 26). His serum contained an abnormal "M" component with an electrophoretic mobility of 2.2–2.4. During his first relapse its concentration was 2.8 gm. per 100 cc. (Table I). Bence Jones proteinuria ranged from 20–22 gm. per day. After urethane administration the serum component fell to 2.3–2.5 gm. per 100 cc. and the proteinuria about 50%. Inulin and p-aminohippurate clearance studies at this time showed virtually normal renal function (28).

After urethane therapy had to be suspended late in the course of his illness, the abnormal serum component fell to 1.0 gm. per 100 cc. and the proteinuria to 0.25–0.5 gm. per day (9).

The urinary protein was studied by electrophoresis on two occasions. Over 90% of it was a single component with a mobility of 4.1. When this protein was added to the serum a new boundary was produced, faster in mobility and well separated from the "M" component (Figure 4).

The diffusion rate of the abnormal component in the serum, $D^{w}_{20,w} = 4.9$, was comparable to that of high molecular weight proteins in other patients (Table I). The urinary protein sedimented as one component in the ultracentrifuge. Its molecular weight was calculated at nearly 37,000.

Case 6. J. C., C27868. This patient, whose illness began with spinal cord compression by a plasmacytoma, has been observed for more than three years (9). An abnormal component with a mobility of 1.9 was present in his serum in a concentration as high as 1.4 gm. per 100 cc. No protein was detected in the urine at any time. The renal clearance of infused inulin and p-aminohippurate one year after the onset of his disease was normal (28).

Case 8. C. K., C44556. This 40 year old colored male was admitted to the hospital after four to six months of increasingly severe back pain. On physical examination he was suffering from generalized bone pain, underweight, and acutely ill. A normochromic, normocytic anemia was present with hemoglobin reduced to 8.2 gm. per 100 cc. Marrow from different sites contained 25–90% abnormal plasma cells. X-ray films of the skeleton showed demineralization and multiple osteolytic lesions in the skull, ribs, vertebrae, and proximal femurs. The NPN was 31 mg., calcium 15.0 mg., phosphorus 2.5 mg. and alkaline

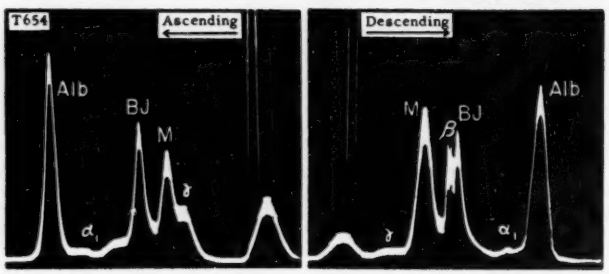

FIG. 4. CASE 4, S. J. C., C28643. PHOTOGRAPH OF ELECTROPHORETIC PATTERN OF MIXTURE OF BENCE JONES PROTEIN AND SERUM
For serum patterns alone see Figure 6 (9).

phosphatase 1.3 Bodansky units per 100 cc. The phenolsulfonphthalein dye was excreted at the rate of 16% in the first half hour and 8% in the second half hour. The specific gravity of the urine after maximal concentration was 1.012. Inulin and p-aminohippurate clearances showed seriously impaired renal function (28).

He died suddenly four weeks after admission. Autopsy showed widespread myeloma. There was precipitated protein in the renal tubules and degenerative changes in the tubular epithelium.

Electrophoretic study of the serum showed an abnormal component with a mobility of 2.4 present in a concentration of 8.6 gm. per 100 cc. The urine contained 0.3 gm. of protein per 24 hours which gave positive Bence Jones coagulation tests. Electrophoretic study of the urine protein showed a mixture of which 70% was a component with a mobility of 4.0 (Figure 7).

Case 9. M. J. W., C35338. This 53 year old woman with chronic back pain of six months' duration had roentgen evidence of severe skeletal demineralization but no anemia (27). The bone marrow contained 11% abnormal plasma cells. The urine contained up to 0.2 gm. protein per day. Inulin and p-aminohippurate clearance studies showed little impairment of renal function (28).

Electrophoretic study of the serum showed an extremely small "M" component, mobility 2.6, present in a concentration of 0.4 gm. per 100 cc. Electrophoretic study of the urinary protein showed a mixture, of which 70% was a globulin component with a mobility of 3.1.

Case 12. C. M. M., C51009. This 77 year old farmer had been ill for five years before his hospital admission on December 29, 1948. His presenting complaints related to destruction of several cervical vertebrae (27). His hemoglobin was 12.8 gm. per 100 cc. The sternal bone marrow contained over 60% abnormal plasma cells. There was no nitrogen retention. The phenolsulfonphthalein dye was excreted poorly, 20% in the first hour and 10% in the second hour. Inulin and p-aminohippurate clearance studies showed severely impaired renal function (28).

Electrophoretic study of the serum showed the smallest recognizable abnormality, a tiny component with a mobility of 2.6 lying between the gamma and beta protein boundaries. The urine contained 1–2 gm. of protein per 24 hours that gave Bence Jones coagulation reactions. Over 80% of the urinary protein on electrophoresis was represented by a globulin component with an average mobility of 3.0 (Figure 7). In the ultracentrifuge the urine protein sedimented as a single component, $S^{\infty}_{20,w} = 3.24$.

Case 27. E. P., C77475. This 51 year old woman had gradually become incapacitated over a period of six to 10 months by low back pain. X-ray films showed pronounced demineralization of the skeleton with innumerable small osteolytic lesions in the ribs, scapulae, clavicles, humeri, vertebrae, pelvis and upper femurs. A chest x-ray taken five years previously during an episode of penumonia showed, on review, definite osteolytic lesions in the ribs. Peripheral blood counts were normal. A bone marrow specimen contained 19% abnormal plasma cells. The blood NPN, serum calcium, phosphorus and alkaline phosphatase determinations and the excretion of phenolsulfonphthalein dye were normal. The urine contained up to 0.1–0.2 gm. of protein per 24 hours that gave the coagulation reactions of Bence Jones protein.

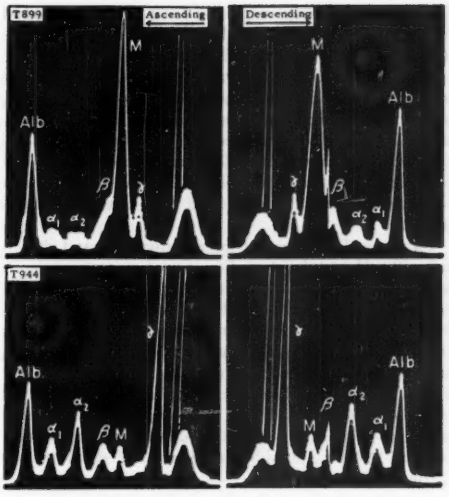

Fig. 5. Photographs of Electrophoretic Patterns of Sera of Patients with Multiple Myeloma Showing More Than One Abnormal Component

T899, Case 32, sharp gamma peak in addition to tall "M" component; T944, Case 38, tall gamma increment and abnormal "M" component.

Electrophoretic study of the serum showed a tiny abnormal component with a mobility of 2.2.

Case 32. B. N., C90306. This 40 year old colored woman had had low back pain for 15 months severe enough to confine her to bed at home for eight months. On examination she was obese and comfortable in bed. There was slight tenderness over the spines of the lumbar vertebrae. X-ray films of her skeleton showed pronounced diffuse demineralization with flattening of the vertebral bodies. A normocytic, normochromic anemia with the hemoglobin reduced to 8.0 gm. per 100 cc. was present. Marrow aspirated from the sternum contained 95% abnormal plasma cells. The urine contained 0.2–0.6 gm. of protein per 24 hours but the coagulation reactions of Bence Jones protein could not be obtained. Phenolsulfonphthalein dye was excreted at a normal rate.

Electrophoretic analysis of the serum showed a large "M" protein component with a mobility of 2.3 present in a concentration of 6.1 gm. per 100 cc. A sharp peak was present in the adjacent gamma protein (Figure 5, T899). The serum was studied in the ultracentrifuge at a concentration of 0.91%. In addition to the major component, which sedimented with a sharp boundary, there were two small heavier components and one lighter. The molecular weight of the major component was calculated at 120,000.

Electrophoretic study of protein from the urine showed that half of it was albumin (Figure 7). A small globulin component, amounting to 18%, had a mobility of 2.3.

Case 33. M. G. K., C92011. This 49 year old white woman had been ill for 10 months with severe weakness, paraesthesias in the extremities and anemia treated symptomatically with blood transfusions. During this time she had lost 16 lbs. in weight. Eighteen years earlier she had been found to have tuberculosis. This was treated with pneumothorax initially, and three years later with thoracoplasty.

On physical examination she was thin, pale and chronically ill. Hematologic studies showed a normochromic, normocytic anemia with the hemoglobin reduced to 8.1 gm. per 100 cc. The white blood count ranged from 9,500 to over 18,000, with a few immature myeloid cells present in the circulating blood. Bone marrow aspirated from different sites contained 10–38% abnormal plasma cells. X-ray films of the skeleton showed demineralization and scattered irregular rarefactions in the ribs, scapulae, humeri, and proximal tibia and fibula. The blood NPN was 63 mg., calcium 11.2 mg., phosphorus 2.8 mg. and alkaline phosphatase 5.3 Bodansky units per 100 cc. There was no detectable excretion of the phenolsulfonphthalein dye.

She became worse rapidly and died three weeks after admission to the hospital. Necropsy showed pulmonary tuberculosis with fibrosis about a cavity in which there were tubercle bacilli, amyloid deposits in small arteries, extensive hyalinization of the renal glomeruli, and protein casts and precipitated calcium in the tubules.

Electrophoretic study of the serum showed a very small "M" component, mobility 2.2, present in a concentration 0.3 gm. per 100 cc. The urine contained 4–5 gm. of protein per day of which 67% on electrophoretic analysis was a homogeneous component with a mobility of 2.3 (Figure 7).

Case 16. W. W. B., C55643. This 48 year old colored laborer, complaining of asthma, was found on routine urinalysis to excrete Bence Jones protein. Examination of the circulating blood showed normal values throughout. The sternal bone marrow contained 15% abnormal plasma cells. X-ray films of the skull, thorax, spine and pelvis showed no bony abnormality. The serum calcium, phosphorus and phosphatase determinations were normal. Inulin and p-aminohippurate clearances showed no impairment of renal function (28).

The electrophoretic pattern of the serum showed no abnormal features. The urine protein amounted to 0.1–0.6 gm. per 24 hours. On electrophoresis over 77% of the urine protein was represented by a globulin component of mobility 3.1. There was no demonstrable albumin.

Case 17. S. H., 93158. This 54 year old colored woman was admitted to the hospital for the treatment of congestive heart failure which developed after some 12 years of arterial hypertension. Bence Jones protein was discovered on routine urinalysis.

Examination of the peripheral blood showed normal values. The bone marrow contained 24% huge abnormal plasma cells. The blood NPN was 34 mg., serum calcium 9.5 mg., phosphorus 2.8 mg., and alkaline phosphatase 3.5 Bodansky units per 100 cc. The serum protein concentration was 6.5 gm. per 100 cc. X-rays of the skull, thorax, spine and pelvis showed no bony abnormalities.

Electrophoretic study of the serum showed no definite abnormality. The urine contained about 1.2 gm. of protein per day. On electrophoretic analysis, 44% was albumin and 43% a component with a mobility of 3.9.

Case 21. L. N., A33105. This 46 year old white woman developed flaccid paralysis of the lower extremities due to collapse of the 6th thoracic vertebra with spinal cord compression (27). Laminectomy was performed and an extradural plasmacytoma partially resected. Examination of the peripheral blood subsequently showed normal values. Marrow from the sternum contained 7% abnormal plasma cells. The blood NPN, serum calcium, phosphorus, and alkaline phosphatase determinations were all normal. The serum protein concentration was 7.2 gm. per 100 cc. Inulin and p-aminohippurate clearances showed moderate impairment of renal function (28).

During her convalescence in the hospital there was no protein in the urine. A few weeks later, when she was again active, proteinuria was consistently present in a concentration of 0.2–0.8 gm. per 24 hours. Electrophoretic study of the serum on two occasions showed no abnormality. Electrophoretic analysis of the urinary protein showed 48% albumin and 25% a homogeneous globulin component of mobility 2.7.

Case 26. J. B., C77930. This 65 year old colored laborer was admitted to the hospital complaining of back pain of 18 months' duration which had been severe enough to keep him from working for 10 months. A tumor of the sternum had been increasing in size for three months and for two to three weeks he had had severe generalized skeletal pain. Physical examination showed a weak, chronically ill, undernourished male with tenderness of the superficial bones who held himself immobile for fear of producing pain. There were nodular deformities over his ribs and sternum. X-ray films of the skeleton showed multiple osteolytic lesions in the skull, ribs, scapulae, clavicles, vertebrae and proximal femora.

Hematologic study showed no anemia but there was a small percentage of immature granulocytes and plasma cells in the circulating blood. Bone marrow from the sternum contained 68% abnormal plasma cells. The urine contained 0.1–0.2 gm. of protein per 24 hours that gave the coagulation tests of Bence Jones protein. The NPN on different determinations was 60–72 mg., serum calcium 17.0–17.5 mg. and serum protein concentration 6.7–7.6 gm. per 100 cc. The serum phosphorus was 4.1 mg. and the alkaline phosphatase 6.2 Bodansky units per 100 cc.

He responded well to urethane therapy. In a few weeks bone tenderness subsided and he had no pain at rest. The nitrogen retention disappeared. He became ambulatory but, seven months after the diagnosis was first made, relapsed and died.

Electrophoretic analysis of the serum before therapy showed no definite abnormality.

Case 34. P. T., C93777. This 58 year old colored laborer had a slight fall and fractured his left femur above

the knee. The fracture site stabilized after being in a cast for two months, but with resumption of activity it broke again. On physical examination he was chronically ill and undernourished. There was a fusiform swelling of the left leg above the knee. X-ray films showed that the fracture passed through a cystic area of bone dissolution about which there was extensive callous formation. In the shaft of the femur above the fracture there were multiple osteolytic lesions. Other large and small destructive lesions were present in the pelvis, vertebrae, ribs and skull. The NPN was 35 mg., serum calcium 15.0 mg., phosphorus 2.3 mg., and phosphatase 7.9 Bodansky units per 100 cc. The serum protein concentration was 6.7 gm. per 100 cc.

The electrophoretic pattern of the serum was normal. The urine contained no protein at any time.

To summarize the findings regarding protein abnormalities in this group of patients with multiple myeloma, abnormal serum components were recognizable in the electrophoretic patterns of 25 of the 30 individuals. In 17 instances the abnormal component migrated as gamma protein, with an electrophoretic mobility of 0.3–1.7, and in eight between gamma and beta protein, with a mobility of 1.9–2.6.

While many sera showed minor and probably non-specific abnormalities in electrophoretic pattern, increase in alpha globulins, irregular variations in beta-lipoproteins, etc., the essential abnormality associated with the proliferation of abnormal plasma cells appeared to be the presence of an unusually homogeneous globulin constituent in the serum. The sera of two patients contained two clearly abnormal constituents each. In Case 32, in addition to a large "M" component, there was a sharp peak in the gamma boundary (Figure 5, T899). In Case 38, a very large gamma component was present and a small "M" component as well (Figure 5, T944).

The quantity of abnormal protein in the serum ranged from the smallest amounts demonstrable by electrophoresis, 0.1–0.5 gm. per 100 cc., to increments as large as 6–9 gm. per 100 cc. Some individuals with little abnormal protein in the serum excreted as much as 10–20 gm. of Bence Jones protein in the urine daily. Others with large amounts excreted negligible amounts or none. In patients with the largest serum increments, however, proteinuria did not exceed 1–2 gm. per day. In individual patients with Bence Jones proteinuria and measurable amounts of abnormal protein in the serum, the amount of protein excreted in the urine remained roughly proportional to the concentration of abnormal protein in the serum as the disease activity was suppressed by therapy and as relapses occurred. Protein was not demonstrated in the urine of five patients at any time. In four of these the amount of abnormal protein in the serum was considerable, 1.0–3.9 gm. per 100 cc. Two patients with disease of widespread anatomic extent had no demonstrable abnormality in the electrophoretic pattern of their sera and minimal or no proteinuria.

In 17 individuals the electrophoretic mobility of the abnormal serum increment could be compared with that of the Bence Jones urinary protein they excreted. In nine instances the mobilities were virtually the same. In five patients the mobility of the urinary protein was somewhat greater than that of the component in the serum, and in three others it was much greater.

That the latter differences in electrophoretic mobility were significant, considering the effect of different media and the presence of other proteins (29, 30), was demonstrated by mixing serum and urinary proteins. In Case 7 the mobility of the abnormal serum and urinary components was virtually the same, 1.4 and 1.5. Protein from the urine was added to the serum and on electrophoresis both components moved together. In Cases 1, 29, and 4, the mobility of the protein from the urine was more rapid than that of the abnormal component in the serum. After Bence Jones protein was added to the sera, the two abnormal components separated clearly on electrophoresis, the urinary protein retaining a faster mobility (Figures 1, 3 and 4). Finally, Bence Jones proteins of different mobilities, from different patients, were mixed together and subjected to electrophoresis. Urinary proteins from Cases 1, 7 and 29 had calculated mobilities of 3.8, 1.5, and 1.6, respectively. Approximately equal quantities of these proteins were mixed together. On electrophoresis, the two of slower mobility moved as one component, and did not separate after twice the usual time allowed for migration, in spite of the great difference in molecular weight (Table I), but separated sharply from that of faster mobility (Figure 6, T937). A mixture of approximately equal amounts of Bence Jones protein from Cases 1,

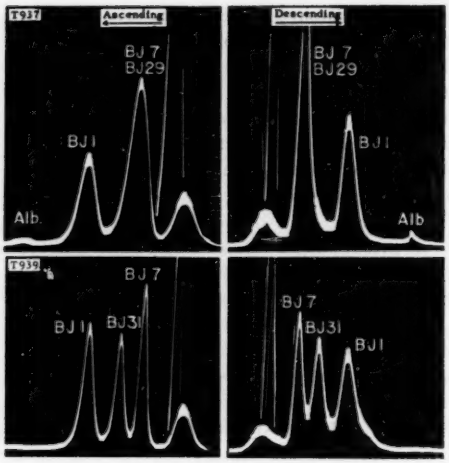

FIG. 6. PHOTOGRAPHS OF ELECTROPHORETIC PATTERNS OF MIXTURES OF BENCE JONES URINARY PROTEINS WITH THE SAME AND DIFFERENT MOBILITIES

T937, protein from Cases 1, 7 and 29; T939, protein from Cases 1, 7 and 31.

7, and 31, the latter having a mobility of 2.5, was subjected to electrophoresis. Each of the three proteins separated sharply (Figure 6, T939).

Although Schlieren methods for measuring diffusion are of somewhat limited accuracy, the $D^{\infty}_{20,w}$ of abnormal gamma components present in high concentration in the sera of eight patients ranged from 3.1–5.0, with an average of 4.2. In two sera with "M" increments, $D^{\infty}_{20,w}$ = 4.9 and 5.0. The diffusion rate of human serum albumin under the same conditions was 6.8 ± 0.4.

Abnormal gamma components separated from the sera of 13 patients were studied in the ultracentrifuge on 16 occasions. Normal gamma globulin was presumably present in many of these fractions but there was no differentiation in the ultracentrifuge. The sedimentation velocities were relatively uniform, $S^{\infty}_{20,w}$ = 6.19 to 6.76 with an average of 6.46. The majority of the molecular weights calculated from the diffusion and sedimentation measurements were from 140,000 to 160,000. One gamma component appeared to have a molecular weight of 130,000 and another over 200,000. The molecular weight of an "M" component was calculated at 120,000.

The diffusion rates of Bence Jones urinary proteins varied considerably, $D^{\infty}_{20,w}$ = 4.7 to 9.8. The sedimentation velocity in the ultracentrifuge varied likewise, $S^{\infty}_{20,w}$ = 2.44 to 4.40. The calculated molecular weight of the Bence Jones proteins excreted by 10 patients was 24,000 (one patient), 32,000–42,000 (four patients), 45,000–52,000 (three patients), 60,000 (one patient), and 90,000 (one patient). In two additional instances, the sedimentation rate would suggest a molecular weight in the range of 32,000–42,000 and in one other about 60,000.

Considering the data relating to renal disease, five of the 30 patients had nitrogen retention before therapy. Three responded well to urethane, and in a few weeks the blood NPN became normal. Inulin and p-aminohippurate clearance studies, carried out in 15 patients, and tests of total kidney function showed severely impaired function in four who did not have azotemia. A correlation between the degree of

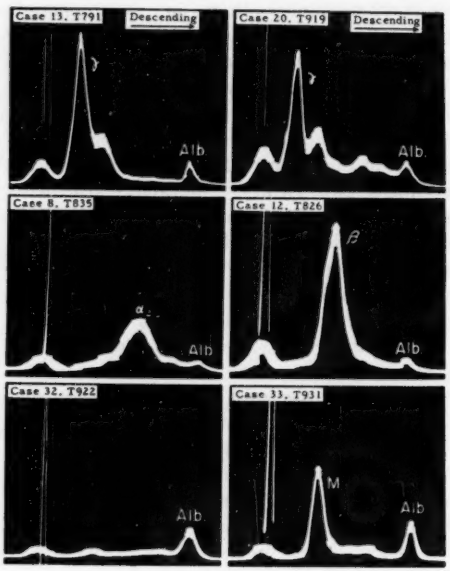

FIG. 7. PHOTOGRAPHS OF ELECTROPHORETIC PATTERNS SHOWING MIXTURES OF PROTEIN IN THE URINE OF PATIENTS WHO, WITH THE EXCEPTION OF CASE 32, HAD EVIDENCE OF RENAL FUNCTION IMPAIRMENT

renal functional impairment and *1*) the amount of abnormal protein in the serum, or *2*) the amount of Bence Jones protein excreted in the urine, its electrophoretic mobility, or its molecular weight, was not apparent. Impaired renal function occurred in patients whose urinary protein was almost exclusively Bence Jones type, but in Case 7 about 15–20 gm. of this protein had been excreted in the urine each day for nearly four years yet the renal clearances remained normal. Nitrogen retention developed in Case 26 before treatment was started. The electrophoretic pattern of his serum was not abnormal, and proteinuria did not exceed 0.1–0.2 gm. per day. Renal clearances were moderately impaired in Cases 14 and 18 in whom proteinuria was never detected. Patients with the greatest impairment of renal function were generally the sicker ones, and their urine contained a mixture of proteins, including albumin, in addition to the Bence Jones protein (Figure 7).

Eleven of the gamma components which had been separated electrophoretically from the sera were tested for precipitation at a pH of about 5.5 when heated to 59° C. and for solubility at 100° C. when acidified further with acetic acid and nitric acid. Gamma protein from three sera showed slight precipitation at the low temperature, but none dissolved well at boiling. All of the Bence Jones urinary proteins, with one exception, showed heavy precipitation below 59° C. when tested similarly. Slightly over half of them dissolved well at 100° C.

DISCUSSION

The results of the above studies are in general agreement with early investigations of the physico-chemical properties of the abnormal proteins in multiple myeloma, thoroughly reviewed in 1948 by Gutman (12). The serum increments vary in amount from those too small to appear in electrophoretic patterns of whole serum, up to 7–9 gm. or more per 100 cc. They range in electrophoretic mobility from slower than the average for gamma protein to at least as fast as that of beta. The abnormal serum proteins produced by different individuals with the disease thus vary in their net surface charge. The components of slow mobility have a molecular weight comparable to that of normal gamma globulin. Those of more rapid mobility, studied in a few instances, may be of smaller molecular size.

Although the molecular weight of Bence Jones urinary protein is generally given as 35,000–40,000, one of the first three specimens studied had a value of about 70,000 (24). Subsequent measurements of sedimentation and diffusion rates have indicated a considerable variation in the size of the protein molecule in different cases (5, 31, 32). The molecular weight of the Bence Jones proteins excreted by our patients, usually between 30,000 and 60,000 with extremes of 24,000 and 90,000, is probably indicative of the extent of the variation in a representative group of individuals with this disease.

The extent to which the abnormal serum constituents in multiple myeloma represent Bence Jones proteinemia was investigated by Gutman and collaborators (4, 5, 12). It appeared that only a very small proportion of the serum increments, in many if not the majority of cases, was due to this protein. By adding Bence Jones protein to sera they were able to augment or introduce serum constituents of beta mobility or intermediate between beta and gamma. The electrophoretic mobility of their Bence Jones proteins ranged from 1.4 to 3.1, generally faster than that of the abnormal serum component in the same patient. They did not regard the differences between the mobility of the serum increments and that of the Bence Jones protein excreted as significant. In their Case 11, a large serum component with a mobility between beta and gamma protein sedimented in the ultracentrifuge at 4.0 S., as compared to that of the Bence Jones protein sedimentation rate of 2.8. Their Case 41 did not have Bence Jones protein in the urine on repeated examinations, but had a large beta increment in the serum. When prepared for study in the ultracentrifuge, the sedimentation rate of the major serum component was slow, 3.0. On dilution of the serum a protein not completely precipitable by trichloroacetic acid appeared. The findings suggested that the serum increment in this instance was a protein complex dissociable on dilution.

Our observations regarding the electrophoretic behavior of the abnormal serum and urinary

proteins, separately and in mixtures, indicate that the Bence Jones protein excreted by a given patient has either the same, or a significantly greater, electrophoretic mobility than the anomalous serum constituent. If the serum and urinary proteins from a given patient had the same mobility, the electrophoretic pattern of the serum would give no information, of course, as to the possibility of Bence Jones proteinemia. If the mobilities were appreciably different, as occurred in nearly half of our cases, a significant quantity of Bence Jones protein in the serum should produce a second abnormal constituent of faster mobility. These were not found.

The suggestion was made long ago that molecular size is important in determining whether or not abnormal proteins are retained in the serum or are excreted in the urine (2). Bence Jones proteins fall within the range of those readily filterable through the glomeruli (17, 18). They may be found in the urine with or without albumin, which has a molecular weight of about 70,000 (33). It is of interest that in our Case 29, a Bence Jones protein with a molecular weight estimated at 90,000 was present in the urine in a concentration 30 times that of albumin. Protein increments retained in the serum appear to have a molecular weight of 120,000 or more (2, 3, 5, 12, 31, 34–36).

The relation of the abnormal serum components in multiple myeloma to Bence Jones urinary protein can be explained by a simple hypothesis consistent with our existing information. The proliferating plasma cells apparently produce a protein of abnormal homogeneity and of high molecular weight. Bence Jones proteins are abnormally homogeneous also, and their molecular weight is one-third to one-sixth that of the serum increments. As plasma cell growth in multiple myeloma is suppressed by urethane, and the concentration of abnormal protein in the serum is reduced, the quantity of Bence Jones protein excreted in the urine decreases or may virtually disappear. The opposite changes occur during relapse. Although the plasma cells could conceivably synthesize more than one variety of protein simultaneously, it seems more plausible that Bence Jones proteins are derived from the serum increments, as a fraction of the larger molecules filterable through the glomeruli. The faster electrophoretic mobility of some of the urinary proteins would indicate an increase in surface charge coming about during the process of fragmentation. Where and how his occurs is a matter for further study. Multiple abnormal serum components, as illustrated in Figure 5, might result from the accumulation in the serum of protein molecules of different mobility, still too large to be readily excreted into the urine. The great variation in the amount of abnormal protein demonstrable in the serum, or present in the urine, in individual patients indicates a considerable difference in either the rate these proteins are synthesized, or in the rate they are broken down. Fragmentary knowledge suggests a rapid synthesis and short survival (37, 38).

The central phenomenon in multiple myeloma is abnormal plasma cell proliferation. In a high percentage of cases, but not invariably, this is accompanied by the appearance of abnormal proteins in the serum and/or urine. The type of protein abnormality varies from patient to patient but remains qualitatively constant once it has developed (4, 9). The outstanding property so far recognized that differentiates normal from abnormal proteins in this disease is the remarkable homogeneity of the latter (39).

Regarding the renal complication of myeloma, it appears to be reversible in some instances, and ordinarily non-progressive in those who respond well to therapy. It appears to occur in patients who are severely ill with the disease, rather than as a result of the excretion of abnormal protein (28).

CONCLUSIONS

1) The abnormal serum and urinary proteins in 30 patients with multiple myeloma were studied, many during different stages of disease activity, by methods including electrophoresis and ultracentrifugation.

2) Abnormal serum components, recognizable in the electrophoretic patterns of 25 of the 30 patients, varied from minute increments to as much as 7–9 gm. per 100 cc.

3) The electrophoretic mobility of the serum increments, 0.3–2.6, ranged from slower than that of gamma to that of beta protein, while the electrophoretic mobility of Bence Jones proteins,

1.2–4.1, ranged from that of gamma to alpha$_2$ globulin.

4) A comparison of the electrophoretic mobility of the abnormal serum increments in 17 patients with that of the Bence Jones urinary protein they excreted, showed virtually the same mobility in nine instances, and a more rapid mobility for the Bence Jones protein in eight instances. Electrophoretic study of mixtures of urinary protein and serum showed that these differences in mobility were significant.

5) Although the concentration of abnormal protein in the serum remained proportional to the amount of Bence Jones protein excreted in the urine in individual patients during fluctuations in disease activity, some patients had large quantities of abnormal protein in the serum, and little or no proteinuria, and vice versa.

6) Abnormal serum components had diffusion rates of $D^{\circ}_{20,w} = 3.1–5.0$ (in 10 patients) and sedimentation velocities of $S^{\circ}_{20,w} = 6.17–6.76$ (in 14 patients). Calculated molecular weights ranged from 120,000 to 200,000 with the majority between 140,000 and 160,000.

7) Bence Jones urinary proteins varied in diffusion rate, $D^{\circ}_{20,w} = 4.7–9.8$, and sedimentation rate, $S^{\circ}_{20,w} = 2.44–4.40$. The calculated molecular weight of Bence Jones proteins excreted by 10 patients was 24,000 (1 patient), 32,000–42,000 (4 patients), 45,000–52,000 (3 patients), 60,000 (1 patient), and 90,000 (1 patient).

8) Available information is compatible with the hypothesis that the small sized Bence Jones urinary proteins may be derived from the serum increments of high molecular weight.

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EVALUATION OF RENAL RESISTANCES, WITH SPECIAL REFERENCE TO CHANGES IN ESSENTIAL HYPERTENSION [1]

By DOMINGO M. GÓMEZ

(From the Department of Physiology, New York University College of Medicine, New York, N. Y.)

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We have elsewhere (1) treated the theoretic aspects of renal hemodynamics at some length, and there presented methods for the evaluation of the physical characteristics of the renal vascular tree. Unfortunately the application of these methods requires precise knowledge of certain variables not available at the present time. It is, however, possible by means of warrantable, simplifying assumptions to effect such simplification in the analytic approach as to make possible the calculation of the major renal resistances from clinically available data. This paper comprises a statement of the necessary assumptions, the equations based thereon, and the calculation of renal resistances in the normal human kidney and in subjects with hypertensive disease.

For practical computation the renal vascular tree can be divided into five functional segments:

A = Afferent vessels
B = Glomerular capillaries
C = Efferent arterioles
D = Peritubular capillaries
E = Venules and veins

If the conditions in the intermediate segments B and D can be specified, the characteristics of the other three vascular segments A, C and E, can be approximately evaluated.

Glomerular and peritubular capillaries. Collating the available data on the nature of the fluid flow in capillaries (2), the relative viscosity of the blood for specified plasma protein concentration (3), and hematocrit (4, 5), the dimensions and number of the glomerular capillaries (6), and the blood flow through the glomerular capillary bed (7), it may be deduced that the glomerular capillaries contribute some 1.8 to 9 per cent of the total renal resistance; *i.e.*, the drop in pressure along the glomerular capillaries is of the order of 1.4 to 7 mm. Hg, a value negligibly small as compared to the drop in pressure across the kidney as a whole (some 80 mm. Hg in normal subjects). This conclusion is supported by the fact that in different species the drop of pressure between the arteriolar and venous end of the capillaries of the systemic circulation ranges from 3 to 20 mm. Hg (8).

As compared with the glomerular capillaries, the peritubular capillaries probably afford an equal or larger cross-sectional area through which the blood moves at a relatively low velocity, and with even a smaller decrement in pressure than in the glomeruli. In the absence of precise information, it may be assumed that the contribution of both the glomerular and peritubular capillary plexus to the total renal resistance is so small that it can be neglected in practical computations.

The elementary mathematical treatment which follows, however, is such that part of the actual resistance of the glomerular capillaries is subsumed in the calculated value of R_A and part in R_E, while part of the actual resistance of the peritubular capillaries is subsumed in R_E and part in R_V. The net effect, therefore, is to introduce a slight, unavoidable but probably negligible error in each of these terms.

For the calculation of the resistances represented by A, C and E, it is required that we know the flow across and decrement in pressure along each segment. The four pairs of values the members of which are to be compared are:

$$(1)\quad R = \frac{P_m - P_v}{Q} \times 1328 = R_A + R'_E + R_V$$

$$(2)\quad R_A = \frac{P_m - P_g}{Q} \times 1328$$

$$(3)\quad R_E = \frac{P_g - P_t}{Q - q} \times 1328$$

$$(4)\quad R_V = \frac{P_t - P_v}{Q} \times 1328$$

Q = afferent renal blood flow
q = filtration rate
both in cc./sec. and corrected to 1.73 sq. m. body surface area.
P_m = mean aortic pressure
P_g = mean glomerular pressure
P_t = mean peritubular capillary pressure
P_v = renal venous pressure
all pressures in mm. Hg
R = total renal resistance
R_A = afferent resistance
R_E = true efferent resistance

[1] Aided by grants from the Knapp Foundation and the Commonwealth Fund.

R'_E = net efferent resistance (to be defined later)
R_V = venular resistance
all resistances in dynes. sec. cm.$^{-5}$

Correction of Q and q to standard body surface area must be made before incorporation into the equations because (*a*) the calculated resistances will thus be automatically corrected for body size as they should be; (*b*) the gross glomerular permeability coefficient $\bar{\lambda}$, as it appears in equation 5, is based upon a filtration rate already corrected for body surface area.

The factor 1328 is obtained by multiplying the weight of 0.1 cc. Hg at 20° C. (sp. gr. 13.546) by the acceleration of gravity (980.6 cm. sec.$^{-2}$), transforming the pressure into absolute units or baryes (dynes. cm.$^{-2}$) which, when divided by flow (cm.^{3}/sec.), gives dynes. sec. cm.$^{-5}$.

Basic premises

I. In speaking of "resistances" as calculated above, it must be recognized, first, that the relation between fluid flow and decrement in pressure in any vascular segment is not a linear one (2, 9–11), and second, that blood viscosity (2, 4, 12) and the force (the parameter, π) of permanent contraction of the smooth muscular fibers of the vascular wall[2] (2, 13–17) complicate the simple concept of "resistance" as a ratio of the decrement in pressure along a vascular segment to the flow. That is, this ratio does not constitute a resistance in the strict sense of Poiseuille's law or Ohm's law; neither does it constitute a conductance in the sense in which this word has been used by the writer (2, 13–16). But in identifying the above ratios as "resistances," we are following widely accepted hemodynamic usage. The chief consequence of this approximation is that "resistance" as here calculated will not vary strictly as the inverse of the fourth power of vessel diameter, as in the simple statement of Poiseuille's law, though changes in the resistance thus calculated will always represent changes in vascular diameter.

II. The following treatment neglects the loss of fluid from the blood as urine, which is not significant except at very large urine flows, and loss of fluid as lymph, the magnitude of which is small but unknown.

III. It is assumed that glomerular plasma flow and peritubular plasma flow represent an identical stream from which glomerular clearance is effected first, followed by tubular clearance and tubular reabsorption.

IV. It is assumed that the glomerular filtrate and the renal interstitial fluid are protein-free.

V. It is assumed that the specific permeability coefficient of the glomerular capillaries is constant in all subjects without glomerular disease, and excluding the limiting conditions discussed below.

VI. It is assumed that glomerular surface area is proportional to body surface area.

Calculation of total renal resistance, R

P_m, P_v and Q are clinically determinable, and therefore equation 1 may be solved directly.

P_m may be measured directly from the brachial or femoral artery, or estimated as $P_d + k(P_s - P_d)$, where P_s and P_d are systolic and diastolic auscultatory determinations and k is a factor[3]

[2] The parameter, π, which has the dimensions of a pressure, has been identified by the writer with the active force of permanent semi-contraction of the smooth muscular fibers of the vascular walls. Together with the internal viscous resistivity of the arterial walls, the parameter π plays a most important role in the dynamic cubic expansion of the arterial reservoir (2). It also constitutes the preponderant factor determining the changes in frictional resistance to blood flow in the small vessels, the caliber of which is essentially defined by the value of this parameter (13–17). With this last connotation the existence of the parameter π has been subsequently recognized by Aperia (18) who, with the designation of "virtual static pressure" which we originally used, has made wide use of it in his hemodynamic studies. The significance of this parameter has been recently stressed again (19). A first attempt has been made to introduce this parameter in renal hemodynamics (1), but further experimental information is needed before such a concept can be used in clinical research (20).

[3] An approximate value of k is given by the formula

$$k = 0.77\gamma + \rho(1 - \gamma)$$

where γ is the ratio of the duration of the systolic ejection phase to the entire duration of the cardiac cycle and ρ is the fraction of the pulse pressure corresponding to the mean value of the amplitude during diastole. Expressed in terms of the pulse rate, N, γ and ρ have the values

$$\gamma = \frac{2.3\sqrt{N}}{60}$$

and

$$\rho = \frac{12(3\gamma + 1)}{100}$$

When N is between 60 and 80, the value of k varies between about 0.390 and 0.45, in agreement with figures given in the literature. These equations are not valid in aortic insufficiency, pulsus alternans, and auricular fibrillation.

averaging 0.44 (21), or by other indirect means (2, 22–28).

P_v may be measured by catheterization of the renal vein, or taken as equal to femoral venous pressure. Where the peripheral venous pressure is not increased, P_v may be taken as 10 mm. Hg on the basis of average normal values. Where the venous pressure is greatly elevated, as in chronic congestive cardiac failure, P_v may be taken as equal to the peripheral venous pressure (29, 30).

Q is the effective renal blood flow as measured by the PAH or diodrast clearance without reference to the extraction ratio.

Calculation of afferent arteriolar resistance, R_A

This calculation involves P_m (*vide supra*) and the mean glomerular pressure, P_g; the latter cannot be measured directly and must therefore be computed. The method of computation which follows is based on the accepted principle that the rate of glomerular filtration is proportional to the difference between the total mean glomerular pressure, P_g, and the sum of the mean oncotic pressure (h) and intracapsular (= interstitial) pressure (H). In the Starling-Cushny hypothesis of glomerular filtration, the glomerular membranes may be conceived to be a uniform filter possessing a fixed permeability to water which is the same in all normal subjects and which can be designated by $\bar{\lambda}$, a gross permeability coefficient corrected for body surface area. Hence

$$q = \bar{\lambda}(P_g - h - H) \quad (5)$$

Equation 5 is derived as follows: if q' is the filtration rate uncorrected for surface area, and λ the gross permeability coefficient in any individual,

$$q' = \lambda(P_g - h - H) \quad (6)$$

but,

$$\lambda = \epsilon S_g \quad (7)$$

where ϵ is the specific glomerular permeability coefficient (cc./sec. per unit glomerular surface area per unit effective glomerular pressure) and S_g is the total glomerular surface area. Where renal function is corrected to standard body surface area, we may designate these quantities by $\bar{\lambda}$ and $\bar{S}_g$, so that

$$\bar{\lambda} = \epsilon \bar{S}_g \quad (8)$$

Dividing (7) by (8),

$$\lambda = \frac{S_g}{\bar{S}_g}\bar{\lambda} \quad (9)$$

Under premise VI, glomerular surface area is taken as proportional to body surface area, S, and consequently

$$\lambda = \frac{S}{\bar{S}}\bar{\lambda} \quad (10)$$

Substituting 10 in 6,

$$q' = \bar{\lambda}\frac{S}{\bar{S}}(P_g - h - H) \quad (11)$$

Since by definition $q = q'\frac{\bar{S}}{S}$

$$q = \bar{\lambda}(P_g - h - H) \quad (5)$$

In obtaining the normal value of $\bar{\lambda}$, we must assign a value to the mean glomerular pressure, P_g, applicable to the statistical average normal subject. Winton's (31) indirect estimates in the dog indicate that the glomerular pressure is about two-thirds of the mean aortic pressure; taking the latter as 90 mm. Hg in normal subjects under standard conditions, we assign a value to P_g of 60 mm. Hg.[4] By taking q as 2.167 cc./sec. (130 cc./min.) (32), and h and H as 25 and 10 mm. Hg on the basis of average normal values, $\bar{\lambda} = 0.0867$.

Rearranging equation 5,

$$P_g = h + H + \frac{q}{\bar{\lambda}} \quad (12)$$

Observations on the filtration rate, pelvic and interstitial pressures in dogs indicate that so long as the urine flow does not exceed 10 per cent of the filtration rate, the pressure in Bowman's capsule must be practically identical with the interstitial pressure (20). Consequently we utilize interstitial pressure, H, in equation 12 as a substitution for intracapsular pressure.

We have shown elsewhere (1) that the conditions determining interstitial pressure are such that, for wide variations in all renal resistances,

[4] The selection of this value in order to define the properties of the normal glomerular membranes is arbitrary and might be designated as an additional premise. Actually, the value of P_g within the range 55 to 65 mm. Hg assumed in calculating $\bar{\lambda}$ has only a moderate influence upon the calculated value of R_A, R_E and R'_E, and no influence upon R_V.

this pressure is predominantly dependent on renal venous pressure, P_v.

The interstitial pressure, H, surrounding the renal venous network is properly defined by the relation

$$(13)\quad H = \frac{2K + aP_v + aK + a\sqrt{(P_v - K)^2 + \pi^2(1 + a)}}{2(a + 1)}$$

with

$$(14)\quad K = (P_m - h_o) - \frac{a}{2}(h_o + \sqrt{h_o^2 + \pi^2})$$

where h_o is the minimum oncotic pressure, K is a lower limiting pressure mentioned below, and a is a coefficient given by the product of the resistance from the aorta to the peritubular capillary network multiplied by the maximum conductance of the venous system (20). The symbol π, which has the dimensions of a pressure, is a parameter depending on the thickness of the vascular walls. When the latter is small, π is also small and the interstitial pressure varies as a function of the venous pressure as indicated above.

The renal interstitial pressure is also approximately defined (1) by the formula

$$(15)\quad H = \frac{R_V P_m + (R_A + R'_E)P_v}{R_A + R'_E + R_V} - h_o$$

in which each symbol is defined in the text. When the venular resistance R_V is considered as being passively influenced by the difference between the intravascular blood pressure and the extravascular pressure, H, the statement made in the text continues to hold within certain limits.

The above equations, however, contain renal characteristics for which values are not directly ascertainable, and consequently we must at present use the approximation method of relating H to P_v.

In theory, it may be expected that interstitial pressure will have a minimal value which is independent of P_v when the latter is below a limiting value, K, but to increase with and approach P_v where the latter is above this limit. This relationship has been confirmed experimentally (33, 34) in dogs and other mammals, the data showing that H has a value of about 10 mm. Hg so long as P_v is normal, and approaches P_v rapidly as the latter is increased.

Hence H is taken as 10 mm. Hg for normal renal venous pressures and as equal to the renal venous pressure when the latter is greater than 10 mm. Hg.

The oncotic pressure, h, can be calculated from the mean plasma protein concentration, C_m, in the glomerular capillaries,[5] which in turn can be calculated from C_A, the concentration in the afferent plasma (in gm./100 cc.), the renal plasma flow, Q′, and the filtration rate, q, or alternatively from the filtration fraction, $F(= q/Q')$:

$$(16)\quad C_m = C_A \frac{Q'}{q} \ln \frac{Q'}{Q' - q} = \frac{C_A}{F} \ln \left(\frac{1}{1 - F}\right)$$

The variation of plasma flow y(x) along the abscissa x in the glomerular capillaries is a complex one (1), but can be simplified to the practically linear relation

$$(17)\quad y(x) = Q' - q\frac{x}{L}$$

in which L is the length of the glomerular capillaries. If C(x) is the protein concentration at any point, and C_o the initial concentration, one necessarily has everywhere

$$(18)\quad C(x)y(x) = C_oQ'$$

and, therefore,

$$(19)\quad C_m = \frac{1}{L}\int_o^L \frac{C_oQ'}{y(x)}\,dx = \frac{C_oQ'}{L}\int_o^L \frac{dx}{Q' - q\frac{x}{L}}$$

By performing the quadrature indicated here, one obtains equation 16. Alternatively one may take the arithmetic mean between C_A and C_E, the latter being the efferent protein concentration as measured by

$$(20)\quad C_E = C_A \frac{Q'}{Q' - q} = \frac{C_A}{1 - F}$$

and one obtains:

$$(21)\quad C_m = C_A \frac{Q' - q/2}{Q' - q} = C_A \frac{1 - F/2}{1 - F}.$$

Equations 16 and 21 give the same result for small values of the ratio q/Q', as shown by expanding in a power series the logarithmic term in 16 and the denominator in 21, neglecting all the terms of higher order than the first.

To relate C_m to oncotic pressure, we have used the empirical relationship

$$(22)\quad h = \alpha(C_m - \beta)$$

where α and β are numerical constants, the first having the dimensions of a pressure. From available data (35–39), when C_m lies between 5 and 8, α has a value of 5 mm. Hg and β has a value of 2. When C_m lies between 8 and 11, α has a value of 7.75 mm. Hg and β has a value of 4. The relationship is applicable, however, only if the albumin/total protein ratio is about 0.60 $\pm$ 0.04.[6]

[5] It is obvious that the effective mean oncotic pressure in the peritubular capillaries will be slightly lower than in the glomeruli. To correct for this factor, however, one has to introduce involved calculations which, in view of the approximate nature of all equations pertaining to renal hemodynamics, are scarcely worth the elaboration. Our method of estimating h, as well as the method of estimating H, introduce slight errors in R_V, but are without significant effect on R_A and R_E.

[6] The oncotic pressure can be related to plasma protein concentration and albumin total protein ratio by a more elaborate and precise formulation (20), but this need not be attempted here. The approximate relations given above are derived empirically but they can be justified by

After calculation of P_g by determination of h and H in equation 12, substitution in equation 2 yields R_A.

True efferent arteriolar resistance, R_E

It must be noted that a quantity, q, of fluid is shunted around the efferent arterioles by filtration and tubular reabsorption of water. We must therefore distinguish between the true efferent resistance, R_E, were this shunting absent, and the net efferent resistance, R'_E (*vide infra*), as calculated with due allowance for the shunt.

The calculation of R_E requires knowledge of the mean glomerular pressure, P_g, which is obtained as described above, and P_t, the mean pressure in the peritubular capillaries, which again must be estimated indirectly. In approaching the calculation of P_t, it may be noted that the permeability coefficient, λ', of the peritubular capillary bed must be extremely large as compared to that of the glomerular capillaries, $\bar{\lambda}$. If the ratio $\lambda'/\bar{\lambda}$ were near unity, the blood pressure at the midpoint of the peritubular capillaries would have to approximate the value of the renal venous pressure, as can be shown by writing two equations, one, *23*, for filtration, q, and one, *24*, for reabsorption, $\bar{\bar{q}}$:

$$(23) \qquad q = \bar{\lambda}(P_g - H - h)$$

$$(24) \qquad \bar{\bar{q}} = \lambda'(H + h - P_t)$$

If we assumed that $\lambda' = \bar{\lambda}$, and writing $q = \bar{\bar{q}}$, it would follow that

$$(25) \qquad P_t = 2(h + H) - P_g$$

Substituting 60 for P_g and 35 for $H + h$, P_t would equal 10 mm. Hg, a value no greater than the known renal venous pressure. This condition could exist only if the resistance to flow in the renal venules and small veins were practically zero, whereas it is well demonstrated (8) that an appreciable decrement in pressure occurs distal to the venous side of the capillary network; assuming that the renal venous system does not differ from the systemic venous system, it follows that P_t must be assigned a value greater than P_v by a difference amounting to the fall in pressure across the venular resistance R_V, which, by equation 4, is $R_V Q$. Consequently, $\lambda'/\bar{\lambda}$ must be greater than 1.

Approaching the question from another point of view, the volume of blood contained in the kidney is relatively large (we estimate this volume to be of the order of 15 to 30 per cent of the total kidney volume) and there is therefore no reason to suppose that the total capillary surface area per unit of blood is any less than in the systemic capillaries. It has been estimated that in the horse, dog and frog, 1 cc. of systemic blood is exposed to a capillary surface area of 7,300, 5,600 and 2,700 sq. cm., respectively (40, 8). Translating the above figures directly, and assuming a weight of 150 gm. per kidney, the total capillary surface area of the two kidneys would be of the order of 225,000 to 450,000 sq. cm. The surface area of the glomerular capillaries in man is variously estimated to be 15,600 (6, 40) and 7,600 sq. cm. (41). If the specific permeability coefficients (per unit surface area) were the same for both networks, the ratio $\lambda'/\bar{\lambda}$ would be of the order of 15 or 60 to 1. The pressure required to effect the separation of the glomerular filtrate is scarcely more than $(P_g - h - H)$ or 25 mm. Hg; to reabsorb this same quantity of fluid through a capillary plexus 15 to 60 times as large would require proportionately less pressure, or about 0.4 to 2 mm. Hg. Consequently P_t may be taken as no more than 2 mm. Hg below $(h + H)$ and the difference may be less.

From equation 24, writing $\bar{\bar{q}} = q$ and neglecting the urine flow,

$$(26) \qquad P_t = h + H - \frac{q}{\lambda'}$$

If, as argued above, λ' is many times greater than $\bar{\lambda}$, the term q/λ' becomes small, as compared to the actual values of $H + h$, and equation 26 approaches the equality

$$(27) \qquad P_t \approx h + H$$

By eliminating $h + H$ between equations 23 and 26, one has

$$(28) \qquad q = \frac{\bar{\lambda}\lambda'}{\bar{\lambda} + \lambda'}(P_g - P_t)$$

where $\frac{\bar{\lambda}\lambda'}{\bar{\lambda} + \lambda'}$ is equal to the compound total permeability of glomerular and peritubular

a formal expansion in Taylor's series of the theoretical expression around selected values of protein concentration and albumin/total protein ratio.

capillaries. Since $\lambda' \gg \bar{\lambda}$,

$$(29) \qquad \frac{\bar{\lambda}\lambda'}{\bar{\lambda} + \lambda'} \approx \bar{\lambda}$$

and *28* becomes

$$(30) \qquad q \approx \bar{\lambda}(P_g - P_t)$$

From *3* and *30* one obtains

$$(31) \qquad R_E = \frac{q}{\bar{\lambda}(Q - q)} \times 1328$$

Net efferent arteriolar resistance, R'_E

In some instances it may be desirable to calculate the true efferent resistance as a distinct feature of renal function. It will be noted, however, that the glomerular filtrate which is reabsorbed by the peritubular capillaries contributes to the total renal resistance and must somehow be subsumed in equation 1. It could appear as an independent term but it seems preferable to subsume it in the efferent resistance, and for practical purposes to calculate the net or compound parallel resistance presented by the true efferent resistance and the resistance presented by filtration and reabsorption.

This net resistance, R'_E, is given by the total flow, Q, and the decrement in pressure between the glomerular and peritubular capillaries,

$$(32) \qquad R'_E = \frac{P_g - P_t}{Q} \times 1328 = R_E \frac{Q - q}{Q}$$

Venular resistance, R_V

Substituting equation 27 in equation 4,

$$(33) \qquad R_V = \frac{H + h - P_v}{Q} \times 1328$$

Renal shunts and uncleared blood

In the foregoing analysis we have treated the problem as though the clearance method directly gave the total renal blood flow, without reference to the extraction ratio, E_{PAH}. However, only some 92 per cent of the renal arterial blood is presented to the renal parenchyma for clearance. The other 8 per cent is presumably by-passed through perirenal fat, the renal capsule, the calyces, pelvis and other non-excretory tissue (31). Conceiving this uncleared blood as comprising an integrated "uncleared shunt" around the active renal parenchyma, the flow of blood through this shunt is

$$(34) \qquad \frac{Q(1 - E_{PAH})}{E_{PAH}}$$

where E_{PAH} (or E) is the renal plasma extraction ratio of PAH. The resistance, R_S, of this shunt is

$$(35) \qquad R_S = \frac{(P_m - P_v)E}{Q(1 - E)} \times 1328$$

The resistance corresponding to the effective renal blood flow is that defined in equation 1 as R. R and R_S are in parallel, and their combination from *1* and *35* gives the resistance, R_W, of the whole kidney (cleared and uncleared blood), which is obviously smaller than either R or R_S:

$$(36) \qquad R_W = \frac{RR_S}{R + R_S} = \frac{E(P_m - P_v)}{Q} \times 1328$$

Locus of pressure equilibrium

At some point, a, along the efferent arteriole the value of the blood pressure necessarily comes to equal the sum of the maximal oncotic pressure h′ and the interstitial pressure, H. This point may be represented as a fraction, a, of the efferent arteriolar resistance encountered between the end of the glomerulus, in the sense where filtration ceases, and the beginning of the peritubular capillaries, in the sense where capillary reabsorption begins, this entire length representing the efferent arteriolar resistance, R_E. The maximal oncotic pressure, h′, is given by

$$(37) \qquad h' = \alpha(C_E - \beta)$$

where α and β (*vide supra*) lie within the limits specified in connection with equation 22 and C_E is defined by equation 20.

That portion of the resistance encountered between the glomerulus and the locus of pressure equilibrium is given by the relation

$$(38) \qquad aR_E = \frac{P_g - (h' + H)}{Q - q} \times 1328$$

which divided by equation 3 yields

$$(39) \qquad a = \frac{P_g - (h' + H)}{(P_g - P_t)}$$

In terms of the characteristics of the renal vascular tree and the external forces applied to it, the fraction a is defined by the equation

$$(40) \qquad a = \left\{1 - \frac{R(h_o + \alpha\beta)}{2(P_m - P_v)[1328(1 - \varphi) - \bar{\lambda}R'_E]}\right\}$$

where α and β are the constants defined in equation 22, φ is the hematocrit taken as a decimal. This relation holds within the limits imposed by the restrictive conditions specified above and can be drawn from the equations given in the text.

The resistance, R^A, between the aorta and the locus of pressure equilibrium[7] is

$$(41) \qquad R^A = \frac{P_m - (h' + H)}{Q} \times 1328$$

$$= R_A + \frac{aR_E \times 1328}{\bar{\lambda}R_E + 1328} = R_A + aR'_E$$

[7] These two relations (equations 41 and 42) have been reported previously (42). However, the notations have been changed ($b = aR_E$) and the permeability coefficient of the peritubular capillary network has been considered

while the resistance, R^E, from this equilibrium point to the vena cava at the level of the kidney is

$$(42)\quad R^E = \frac{(h' + H) - P_v}{Q} \times 1328$$

$$= R_V + \frac{(1 - a)R_E \times 1328}{\bar{\lambda}R_E + 1328} = R_V + (1 - a)R'_E$$

Practical applications and limitations

In one important respect the approach outlined above is different from that presented by Smith and associates (43), who assumed that filtration equilibrium is reached in the glomerulus. For such to be the case, most of the renal vascular resistance would have to be concentrated in the glomerular capillaries (which is equivalent to assigning practically zero resistance to the afferent, efferent and venular segments), and the gross permeability coefficient of the glomeruli would have to be as great as or greater than that of the peritubular capillaries. If such were the case, the filtration rate and the interstitial pressure would vary over wide ranges with variations in renal blood flow. The postulate that filtration equilibrium is reached in the glomerulus contradicts many theoretical and practical considerations (1) and is untenable. Lamport (44, 45) has used this assumption, and because the premise is untenable, the equations based thereon are also invalid. Other assumptions enter into his equations which are unsatisfactory, but space does not permit us to discuss them here.

In practical computations, q, Q′ and Q are corrected to standard surface area, C_m is calculated by equation 16 and h is calculated from equation 22. Selecting the appropriate value of H, P_g is calculated by equation 12. P_t is calculated by equation 27. These values are then introduced in equations 2, 3 and 4. R'_E is then calculated from equation 32.

The above equations are subject to the following limitations:

a) Equations 1 to 4 by definition are applicable to clearance data where E_{PAH} (or E_D) can be assumed to be high (90 per cent or above), so that the contribution of uncleared blood to renal resistance can be neglected, and where E_{PAH} (or E_D) is known, the contribution of uncleared blood can be treated by equations 35 and 36. But where E_{PAH} (or E_D) has been reduced by disease the equations by definition are no longer applicable. The question of applicability in the presence of possible renal impairment must be considered on its own merits, with reference to every assumption made, as in the following discussion of renal resistances in patients with essential hypertension.

b) Equations 12 and 27 (and hence equations 2 and 3) should be applied with caution to patients with glomerular disease (acute and chronic glomerulonephritis, glomerulocapillary sclerosis, nephrosis, amyloidosis, etc.) because they involve the assumption that the gross glomerular permeability coefficient has the calculated and idealized normal value, $\bar{\lambda}$. For the same reason, the applicability of

as having a value many times greater than that of the glomerular capillaries.

TABLE I

Renal vascular resistances in normal subjects

P_m indicates the mean arterial blood pressure, 60q, 60Q′, and 60Q, the filtration rate, effective renal plasma flow and effective renal blood flow, respectively, per 1.73 sq. m. body surface area. Resistances are given in c.g.s. units (dynes sec. $cm.^{-5}$). The term a is defined in the text.

No.	Subject	Age	P_m	60q	60Q′	F	60Q	R	R_A	R_E	R_V	a
		Years	*mm. Hg*	*cc./min.*	*cc./min.*	*per cent*	*cc./min.*	*Dynes sec. cm.$^{-5}$*				
1	E. C.	51	90	143	684	21	1242	5126	1434	2085	1779	0.87
2	J. W.	51	93	170	669	25	1218	5432	1022	2696	2111	0.83
3	D. S.	50	80	119	364	33	670	9800	2523	4369	3864	0.68
4	E. D.	27	83	116	666	17	1254	4635	1912	1673	1182	0.88
5	J. B.	50	102	122	548	22	828	8752	3174	2895	3134	0.43
6	P. Q.	36	84	96	827	16	1362	4329	1368	1262	1726	0.86
7	H. K.	23	110	146	624	23	1104	7211	2430	2523	2656	0.80
8	S. M.	31	88	128	612	21	1134	6812	890	2098	2722	0.67
9	A. M.	42	84	106	648	16	1200	4914	1195	1607	2243	0.78
10	F. T.	40	90	90	429	21	762	8366	3240	2231	3134	0.80
11	S. C.	47	105	147	661	22	1116	6786	2456	2523	2138	0.84
12	E. K.	38	100	123	654	19	1086	6640	2576	2138	2138	0.84
13	T. K.	29	88	147	768	19	1320	4714	1102	2085	1766	0.87
14	R. W.	42	85	108	684	16	1092	5471	1766	1819	2058	0.86
15	J. S.	45	94	130	798	16	1242	5392	1872	1939	1779	0.90
16	J. W.	35	80	130	756	17	1374	4064	823	1740	1660	0.86
17	F. F.	31	95	146	520	28	906	7477	1992	3200	2802	0.78
18	E. O.	45	91	97	594	16	900	7171	2868	1992	2523	0.84
19	J. D.	62	98	123	587	20	906	6866	1992	2603	2603	0.82
20	A. M.	36	88	103	563	18	936	6640	2350	2058	2470	0.82
21	W. M.	33	96	122	529	23	978	7012	2961	2377	1965	0.82
22	O. D.	25	92	198	768	26	1410	4635	1938	1806	2390	0.90
Average			91	128	630	20	1098	6281	2005	2258	2240	0.81

TABLE II

Renal resistances in subjects with essential hypertension

Data as in Table I

No.	Subject	Age	P_m	60_Q	$60Q'$	F	60Q	K	R_A	R_E	R_V	a
		Years	*mm. Hg*	*cc./min.*	*cc./min.*	*per cent*	*cc./min.*	*Dynes sec. cm.$^{-5}$*				
1	A. W.	44	193	65	317	21	455	32005	24515	2788	5179	0.67
2	J. D.	45	162	84	297	28	513	23771	16733	2855	5117	0.41
3	T. V.	59	162	83	267	31	496	25099	16866	3360	5312	0.57
4	S. B.	36	158	86	382	23	744	15800	10737	2178	3240	0.71
5	M. R.	61	152	74	346	21	651	20186	13758	2536	4223	0.70
6	J. S.	71	150	74	315	23	501	22310	14688	2868	4834	0.59
7	C. M.	39	146	65	405	16	524	20717	14342	2364	4980	0.75
8	G. M.	50	145	83	459	18	768	13944	9203	2032	3001	0.78
9	M. B.	33	142	98	436	22	738	14210	8805	2536	3240	0.75
10	J. M.	51	142	98	391	25	714	14741	9150	2510	3400	0.72
11	D. T.	55	140	97	328	29	546	18326	11567	3572	3864	0.69
12	H. H.	49	135	100	758	13	1450	6866	4196	1235	1527	0.87
13	L. M.	52	125	106	529	20	846	10836	5976	2377	2776	0.80
14	R. S.	37	124	96	474	21	864	10518	4754	2390	2696	0.82
15	J. R.	47	124	122	459	26	800	11354	5570	3028	3240	0.74
16	I. L.	45	120	105	706	14	1018	8605	3997	1912	2191	0.91
Average			145	89	403	22	721	16790	10900	2520	3670	0.72

these equations to infants in whom the fetal glomerular membranes persist (32), and in senile individuals in whom many glomeruli may be canalized, is inappropriate.

c) For equation 22 to be valid, the systemic plasma concentration of total protein should not be below 5 nor above 8 gm./100 cc. and the albumin/total protein ratio should be between 0.55 and 0.65.

d) The renal blood flow, corrected for body surface area, should be above one-third (400 cc./min.) of its normal value (1200 cc./min.); otherwise the concept of resistance as a simple ratio between flow and pressure (equations 1 to 4 inclusive) will probably become grossly inaccurate.

e) It is improbable that equation 12 will be valid if the filtration rate is markedly reduced (<50 cc./min.), and under these conditions it is less likely that the renal interstitial pressure, H, can be equated with the intracapsular pressure.

f) The filtration fraction should not exceed 33 per cent of the renal plasma flow, because at higher values the approximation equation 27 becomes inaccurate.

g) Cases where the calculated values of P_g are above 75 or below 45 mm. Hg should be considered as of doubtful accuracy because great changes around the median value 60 mm. Hg would imply excessive expansion or contraction of the glomerular membranes and consequently changes in the specific glomerular permeability coefficient.

h) Caution must be used in interpreting the causation of changes in R_A, R_E or R_V. Active constriction of the efferent arterioles, for example, will by reduction of pressure distally elicit passive constriction in the venular system with an increase in R_V, while having converse passive effects upon the afferent arterioles and R_A. There is no way at the present time to distinguish passive from active changes in any of the vascular segments, and the effects of drugs, disease, etc. must be interpreted with this qualification.

Renal resistances in normal subjects under standard conditions

For the calculation of renal resistances in normal subjects under standard conditions we have used data reported by Bolomey and associates[8] and unpublished data collected in this laboratory by Drs. Maxwell, Breed and Fishman. The resistances given in Table I are calculated from the data there recorded using P_v = 10 mm. Hg: where the plasma protein concentration was not available (about half of the cases), this value was assumed to be 7 gm./100 cc. The calculated resistances recorded in Table I require only one comment: the resistance of the venular system, R_V, is significantly large in respect to the total renal resistance, R, and in the average equals the resistance of either the efferent or afferent arterioles.

The value a, representing the fraction of the

TABLE III

Renal resistances calculated from the data of Corcoran, Taylor and Page (47) on subjects with essential hypertension

	P_m	60_Q	60Q	R	R_A	R_E	R_V
Highest	200	152	1459	30215	22284	3744	4878
Lowest	108	71	528	6786	3944	1622	1368
Average	150	99	848	18675	13930	2549	2536

[8] We are indebted to Dr. Lauson, and Drs. Corcoran, Taylor and Page for additional information not included in the published reports (46, 47).

efferent resistance R_E corresponding to the point at which pressure equilibrium is reached, needs no comment at this time, the data supplying a basis of reference in connection with shifts in this value during disturbances in the renal circulation.

Renal resistances in subjects with essential hypertension

The data on hypertensive subjects analyzed here comprise 11 subjects reported by Bolomey and co-workers (46) (Table II), five subjects on whom unreported data were available in this laboratory, and 35 subjects reported by Corcoran, Taylor and Page (47, 48) (Table III). Here again P_v was assumed to be 10 mm. Hg and the plasma protein concentration where not available was taken as 7 gm./100 cc.

It is known that in advanced stages of essential hypertension the extraction ratio of diodrast or PAH may be reduced (49–51) to such an extent that the clearance of these substances falls markedly below the total renal plasma flow. It is also known that the apparent filtration fraction may increase in some subjects to values ranging from 0.30 to 0.50, a circumstance which Goldring and associates (52) attributed to the formation of impotent nephrons in which, despite the persistance of glomerular filtration, tubular extraction is impaired. Whether the two phenomena are related has not been determined, but it is highly probable that in any subject in which renal impairment has progressed to the point where the apparent filtration fraction is above the normal parameter (32) of $m + 2\sigma$, (c. 0.27), assumption III is inapplicable. Consequently we have selected for analysis only those subjects in whom the filtration fraction lies below 0.30. We have furthermore excluded subjects in whom the filtration rate is less than 65 cc./min., or the renal blood flow below 400 cc./min. None of the subjects showed evidence of cardiac failure or specific renal disease.

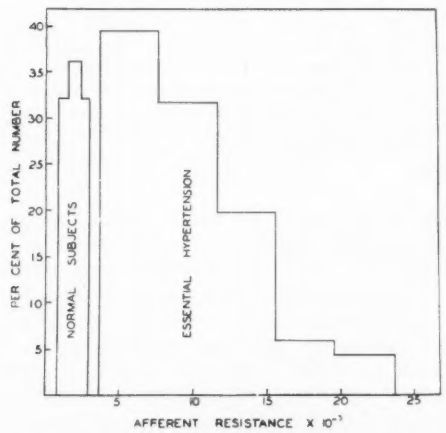

FIG. 1. DISTRIBUTION OF AFFERENT ARTERIOLAR RESISTANCE IN NORMAL AND HYPERTENSIVE SUBJECTS

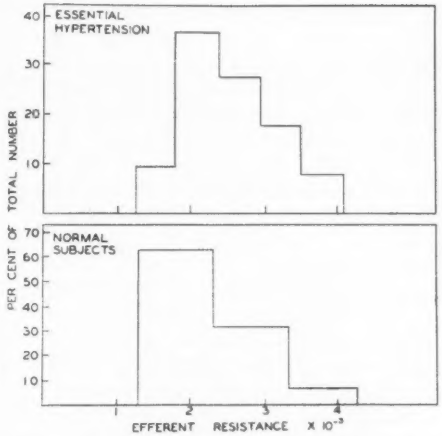

FIG. 2. DISTRIBUTION OF EFFERENT ARTERIOLAR RESISTANCE IN NORMAL AND HYPERTENSIVE SUBJECTS

The results of our analysis of the two series are so similar that they will be considered together in the following discussion and in the figures.

The following points merit specific comment:

a) The frequency distribution of R_A among the hypertensive subjects is compared with the normal distribution in Figure 1. The lowest value of R_A among the hypertensive subjects exceeds the highest normal value.

b) Although R_E (and consequently R'_E)[9] tends to be moderately increased, the mode lies below the upper normal limit in the frequency distribution curve (Figure 2). The data will not enable us to determine whether this increase

[9] R_E rather than R'_E has been given in Tables I, II and III because the latter can be calculated directly as $R - (R_A + R_V)$.

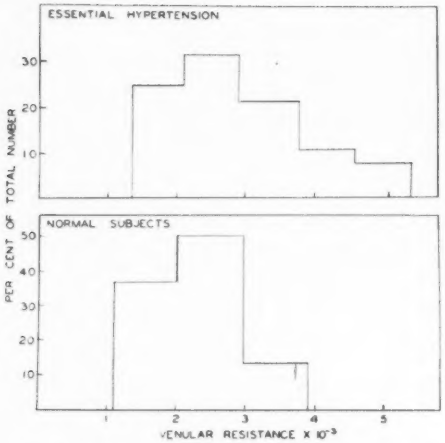

FIG. 3. DISTRIBUTION OF VENULAR RESISTANCE IN NORMAL AND HYPERTENSIVE SUBJECTS

is active or whether it is a passive consequence of the reduction in glomerular pressure.

c) Venular resistance is also-elevated in the mean, but the mode still lies within the upper normal range (Figure 3).

d) Total resistance, R, is increased in nearly all hypertensive subjects, this increase being attributable predominantly to the increase in R_A (Figure 4).

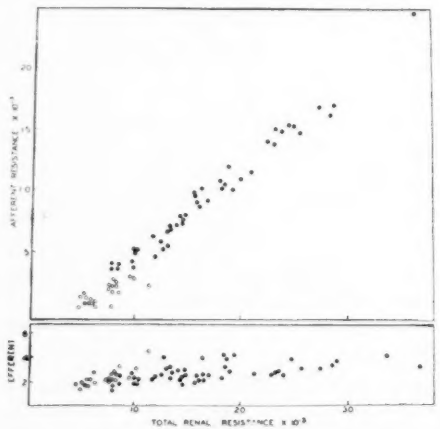

FIG. 4. VARIATIONS OF AFFERENT (ABOVE) AND EFFERENT (BELOW) ARTERIOLAR RESISTANCE WITH THE TOTAL RENAL RESISTANCE IN NORMAL (OPEN CIRCLES) AND HYPERTENSIVE (DOTS) SUBJECTS

e) One might anticipate that the increased afferent resistance would parallel the increase in systemic arteriolar resistance, in which case the renal blood flow and filtration rate would remain unchanged from normal in the face of increased arterial pressure. However, such is not the case; the renal resistance increases relatively more than the systemic resistance. To illustrate this point we note that in the majority of hypertensive subjects reported by Bolomey and associ-

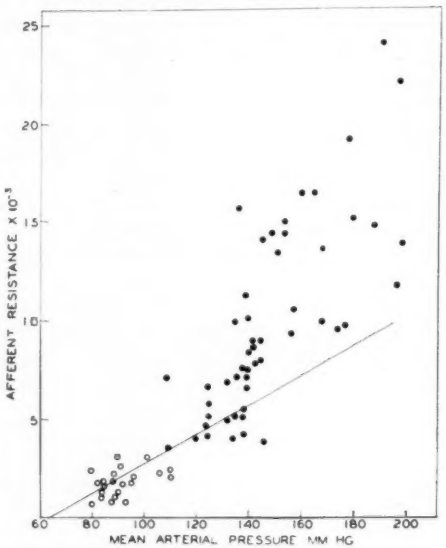

FIG. 5. AFFERENT ARTERIOLAR RESISTANCE IN RELATION TO MEAN ARTERIAL PRESSURE IN NORMAL (OPEN CIRCLES) AND HYPERTENSIVE (DOTS) SUBJECTS

The line indicates the changes in afferent resistance to be expected with increasing arterial pressure if renal blood flow and filtration rate retained the average values observed in the normal subjects represented by the open circles.

ates (our Table II), the cardiac output had the same modality and range as in their control subjects (our Table I). Therefore, the mean arterial pressure may be used as a rough index of the resistance in the systemic arteriolar bed. If in equation 2 we substitute the average normal values of Q (18.3 cc./sec.) as given in Table I and the average value of P_g as calculated from these data (= 63.5 mm. Hg), and solve for R_A as a function of P_m, we obtain the solid line in Figure 5. This line represents the values of R_A re-

quired at increasing values of P_m to yield a constant renal blood flow and filtration rate in the face of increasing P_m. It is clear that with increasing hypertension, there is a relatively greater increase in resistance in the afferent renal arterioles than in the systemic arterioles as a whole. These calculations give more exact expression to the well demonstrated fact that the renal blood flow tends to decrease below the normal value with advancing disease, despite the increase in blood pressure (31).

Our analysis indicates that the consistent and characteristic change in the renal circulation in essential hypertension is in afferent arteriolar resistance, which may be increased several fold: the increase in the efferent arteriolar and venular resistance, slight as it actually is, may be in part attributable to the passive decrease in caliber which results from the resulting decrease in glomerular and post-glomerular pressure.[10]

When Goldring and colleagues (52) concluded that essential hypertension was characterized by increased efferent arteriolar tone (R_E as here defined), they attempted no quantitative analysis of afferent arteriolar or venular tone, and they were led to their conclusion by inadequate analysis of hemodynamic factors, as cited earlier in this paper. Our present study forces us to accept that the increase in R_E as here calculated is at most a slight one; the outstanding change in renal hemodynamics is reflected in the increase in afferent resistance. Our analysis throws no light on whether this increase is anatomical or functional, but the demonstration by Goldring and associates (52) that the renal hyperemia induced in most hypertensive subjects by pyrogen is comparable in degree to that induced in normal subjects is evidence that the predominant factor is functional.

SUMMARY

1. Equations are developed for the calculation of the total renal resistance, R, the afferent arteriolar resistance, R_A, the pure efferent, R_E, and the net efferent arteriolar resistance R'_E (a term that includes the diversion of the glomerular filtrate around the efferent arteriole), and the venular resistance, R_V.

2. The calculations are based upon the clinically determinable variables of mean arterial blood pressure, the renal blood flow and plasma flow, the filtration rate, the protein concentration of the blood and the renal venous (or the femoral) pressure. Assumptions with respect to renal interstitial pressure and other matters pertinent to the validity of the working equations are stated in the text. Some of the limiting clinical conditions under which the working equations are applicable are defined.

3. Calculations are presented for the above resistances in 22 normal subjects who had been examined under standard conditions. A hitherto unrecorded datum is the relatively large magnitude of the renal venular resistance, which in the average equals the resistance of either the efferent or afferent arterioles.

4. Data on 51 subjects with essential hypertension reveal that the predominant change in the renal circulation in this disease is an increase in afferent arteriolar resistance. This increase is relatively greater than the increase in the resistance of the systemic arterioles.

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[10] In view of the above results, the excessive increase in filtration fraction which is so generally characteristic of the disease in its advanced stages should be referred in considerable part to failure of tubular clearance (impotent nephrons) rather than to increased glomerular pressure.

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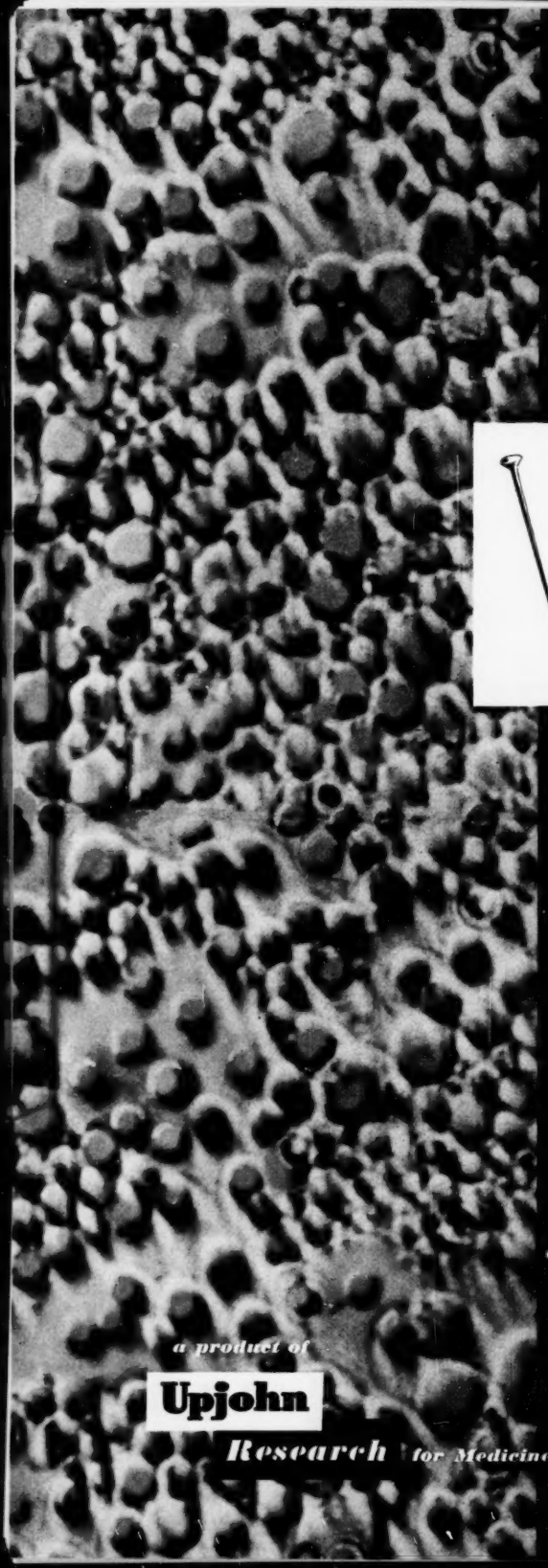

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